

Tag (see P29)

Project No. _____ Appl. No. 09/000,421

Book No. _____

65

age No. _____

Tag assay mix (P557)
except no activated DNA

[A]

(40 Rxns)

0.5 M Tris pH 8.3

100

✓

1 M MgCl₂

4

✓

3 M KCl

33.3 μ l

✓

1 ATGC-TP 10 μ l each

40

✓

2 ³²P dCTP

5.1 4.2 μ l

✓

H₂O

10.2 19 μ l

✓

(1) 385 μ l $\xrightarrow{1.4 \text{ ml}}$ 385

(2) (use 35 μ l / 5 μ l rxn)

(same as P17)

30 μ l 18 500 pmoles / μ l

13.3 μ l

66.7 μ l

e. 11 Rxns
(45 μ l / 5 μ l rxn)

0.165 μ g / μ l

H₂O

96.7

48.5

① = 0.2 μ g DNA / 50 μ l
② = 1 μ g DNA / 50 μ l
(= 5 μ g total / 50 μ l)

48.5

48.5

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

45 μ l $\xrightarrow{\hspace{10em}}$

40 μ l $\xrightarrow{\hspace{10em}}$

total units:

CKBTI

78

5

5

0.4

6

5

5

0.8

25

5

5

1.5

5

5

5

3.125

5

5

6.25

5

5

12.5

5

5

25

5

5

50

5

5

100

5

5

200

4 μ l

50 μ l

2 min at 74°C \rightarrow add 10 μ l EDTA
spot 40 μ l on GFC

To Page No. _____

Used & Understood by me,

Enrica Polansky

Date

10/24/94

Invented by

Recorded by

Date

10-18-94

Sim Page No. _____

	units / 50 μ l			pmol
0.2 μ g DNA	0.4	1	133.00	2.6
	0.8	2	248.00	4.9
	1.6	3	264.00	5.2
	3	4	470.00	9.2
	6	5	633.00	12.5
	12	6	886.00	17
	25	7	991.00	19.5
	50	8	995.00	19.6
	100	9	999.00	19.7
	200	10	883.00	17.4
1.0 μ g DNA	0.4	11	2146.00	42
	0.8	12	3847.00	73
	1.6	13	6695.00	133
	3	14	12077.00	238
	6	15	17179.00	339
	12	16	17333.00	342
	25	17	22279.00	440
	50	18	22941.00	452
	100	19	23863.00	471
	200	20	24510.00	477
	500	21	92.00	
	1000	22	304197.00	
②				76 cpm / pmol

$\sqrt{76 \text{ cpm/pmol}}$

need time course at high and low [Tag]
to see if lag plays a role. In a PCR with
15-30 min elongation time, effect of lag would
be minimized

Results per Tag spot = $100,000 \text{ u/mg}$
25 units = 50 nm Tag
50 μ l

Both plots (0.2 and 1.0 μ g DNA) saturate at 50 nm Tag
suggesting an equilibrium effect of pol DNA binding
rather than titration of pol at 1 pol/mg DNA
saturation at 10 n / 0.42 pmol circles

for 200,000 u/mg Tag

1 unit Tag = 0.053 pmol molecules \sim 1:1 Tag/circle

1 μ g mp19 \Rightarrow $\frac{1 \times 10^{-6} \text{ g}}{(330 \text{ g mole}^{-1}) (7250 \text{ bp})} = 0.42 \text{ pmol circle}$

Page No. _____

Witnessed & Understood by me,

D. S. S. S. S.

Date

10/24/94

Invented by

R. C. R. D. by

Date

10-27-94

Project No. _____
Book No. _____

TITLE Tag - Mutant \rightarrow Heparin Pool over
Super Q GSD

102

From Page No. _____

so Heparin Pool \rightarrow dialyzed against Bfr A \rightarrow 250 mL - 2 exch
~ 12 mL -

Bump 5 mL Super Q GSD column w/ Ga HCl + NaCl \rightarrow

Wash w/ H₂O

Equilibrate w/ Bfr A inlet conductivity 1.37 mS
outlet conductivity 1.42 mS

Sample - 1.5 mS

saved 1 mL of Load material - Load ~ 11.5 mL -
collect Load flow through + wash

Wash w/ Bfr A - Flow rate - 1 mL/min -

Gradient Bfr A \rightarrow Bfr B \rightarrow 10 vts - 50 mL total

collect 1 mL fractions -

Pool 10-12 dialyze against storage buffer -

SDS premix - add 11 μ L hot dCTP -

5 μ L / rxn - 5, 1, 2, 4 μ L - enzyme dilute 1/20

D

DS 1	62892.00	Y ₈₀
1 2	53562.00	
Y ₈₀ 2 3	80556.00	Q Pool
2 4	80834.00	
Y ₈₀ 5	39642.00	Q Pool
1 6	55734.00	
2 7	61384.00	
4 8	69380.00	Y ₈₀
1 9	49764.00	
Y ₈₀ 10	42686.00	Hep Pool -
4 11	75336.00	Q load
1 12	60344.00	
Y ₈₀ 13	50018.00	Hep load -
4 14	57888.00	
15	652.00	

1	1	7260.00	
Y ₃₀₀₀ 2	2	7498.00	129 U/ μ L
3	4	13534.00	117 U/ μ L
Y ₃₀₀₀ 4	1	2836.00	
5	2	4118.00	
6	4	4440.00	

SA₁ = 78 cpm/pmol

Factor = 1.154 x 10⁻⁵

To Page 1

Witnessed & Understood by me, 1/8

Date 2/2/94

Invented by E. H. Hagan

Dat 12/94

R cord d by

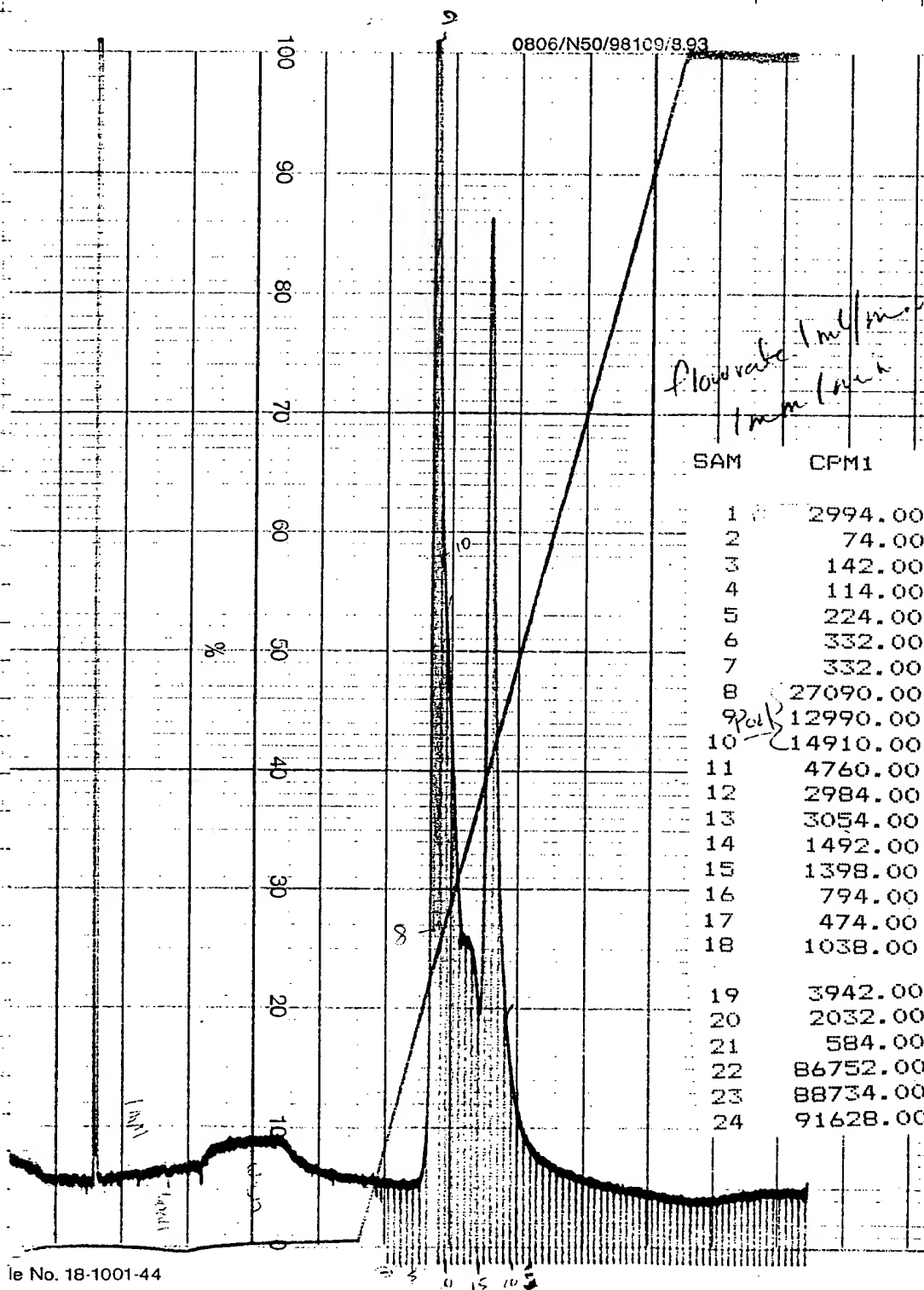
Super Q-650-

Pr j ct No. _____

Book N . _____

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25 ml H₂O
+
1.5 ml sample
↓
74°C - 7 min.

ice - 10% S.M.S. 20A
spin
↓
spot 20 ml GF10
TCA wash.

- ① LM-410
 - ② PT
 - ③ WFT
 - ④ S
 - ⑤ 6.7
 - ⑥ 8
 - ⑦ 9
 - ⑧ 10
 - ⑨ 11
 - ⑩ 12
 - ⑪ 13
 - ⑫ 14
 - ⑬ 15
 - ⑭ 16
 - ⑮ 17
 - ⑯ 18
 - ⑰ 19
 - ⑱ 20
 - ⑲ 21
 - ⑳ 23
 - ㉑ 21
 - ㉒ 22 → 2 μl mix
 - ㉓ 25
- 1/10 dilution

le No. 18-1001-44

To Page No. _____

sed & Understood by me,

jk

Date

2/21/95

Invented by

C. Lynn

Recorded by

Date

12/94

T. neapolitana DLE (2779) SOM

g No. 48

October 13, 1994 (Thursday)

I infected DH12S cells with the ϕ from #5, #6, #7 (2m cells
 grown in 2x YT + 3 μ l ϕ ; grown 37°C (air shaker) 16 hrs)

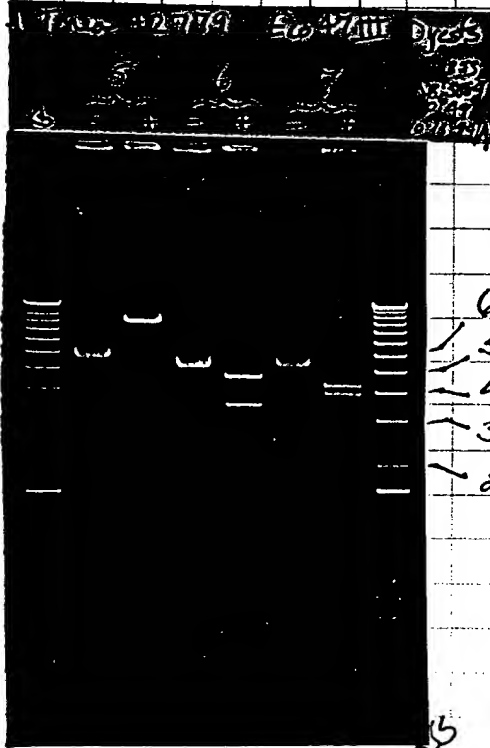
RF isolated by alkaline/SDS except 1 μ l RNase A (1mg/ml) added
 to prep at 10Hq OAc addition

RNA dissolved in 50 μ l T₁₀E₁

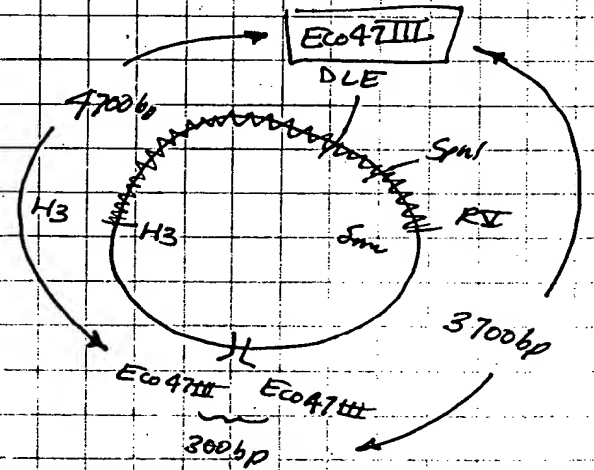
DIGEST SCHEME

(React 3)	HOH	10 μ l	✓	Incubated 37°C (heat block) 1 hour
	10x B/R	2	✓	
	DNA	7	✓	
(4 μ l)	Eco47III	1	✓	
	Total	20 μ l		

3% Agarose Gel (1xTAE); 190V/16



Comments:

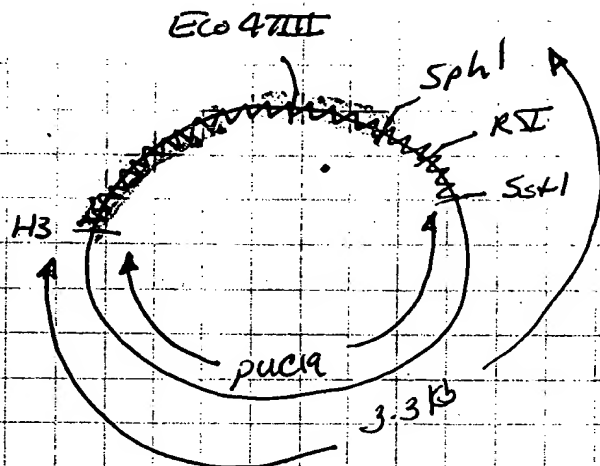
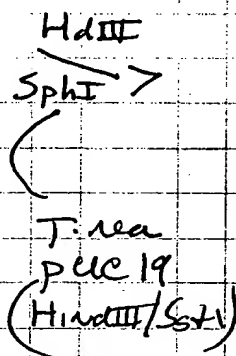
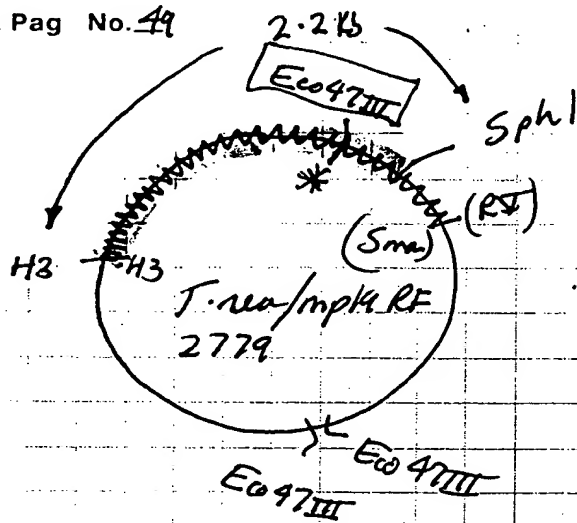


The bands are migrating
 at the expected distances for
 #6. There must have been
 overabundance of some
 "component" causing the DNA
 to run faster. I will clone
 into the pUC vector.

To Page No. 50

Read & Understood by me,	Date	Invented by	Date
May 10/94	10/24/94	Recorded by J. Schmidt	10-13-94

October 13, 1994 (Thurs)



DIGEST SCHEME			#6
		<u>T. nuc / pUC</u>	<u>2729 / mp19</u>
(React 2)	HOM	12 μ l ✓	6 μ l ✓
	10x Bfr	2 ✓	2 ✓
	DNA	4 ✓	12 ✓
(104 μ l)	HindIII	1 ✓	1 ✓
(104 μ l)	SphI	1 ✓	1 ✓
	Term	20 μ l	20 μ l

Incubated 37°C (hot start)
1:10 → 1:50

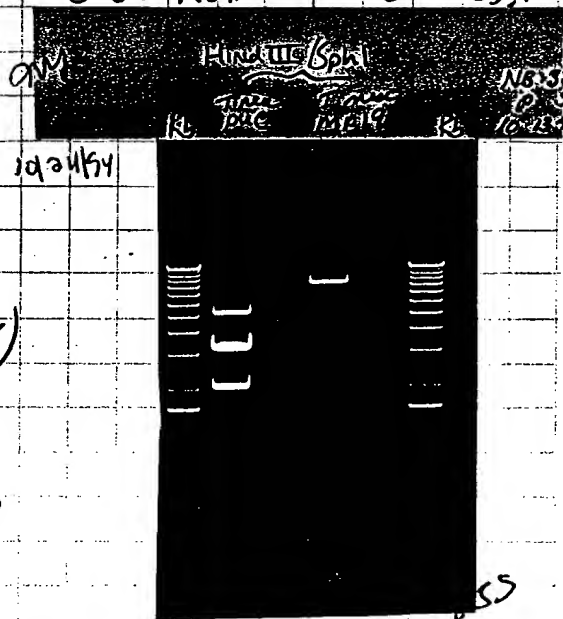
0.8% Agarose Gel (1xTAE); 19C

Comment 6: I should see

a. 3.3 Kb (desired fragment)

and a 2.2 KB fragment from the

T. nea /pUC19 clone and I do. Unfortunately I should see a 7.9 Kb fragment and 2.2 Kb (desired fragment) from the *T. nea* /mp19 (2779 ~~66~~6) RF DNA and I don't. Both sites were present before I performed the mutagenesis (see p. 35) - I will have to repeat.



Project No. _____

Book No. _____

TITLE Deep Vent / GAPDH / diff primers

16

11/16/94

Form Page No. _____

Purpose: Since GAPDH - PCR worked with 3' Thiol primers attempted the same amplification with other available primers, under same conditions.

- Deepvent buffer enzyme at 1U and 0.5U
200 pg Template Mg at 2, 3, 4 and 6 mM
200 μ M dNTP
1 μ M primers

- did just one of each. primers
no do Lac FWD & Lac Reverse (100 μ M)
* 2697 & 2696 " " 3'-1 PPT
+ dU " " (10 μ M)

- each primer set - 10x RX were made.

	Regular	17-20 = 0.5 U	dU	23-36 = 0.5 U	3'-1 PPT	
		21-24 = 1.0 U		37-40 = 1.0 U		25
10x buffer	50		50		50	29
dNTP	10		10		10	
primer 1	5		50		20	1
2	5		50		20	
Template	20 (100 pg/x)		20		20	
H ₂ O	260		270		330	

450 \rightarrow 45 μ L/RX \leftarrow 450 \leftarrow 450

added 5 μ L of	2	3	4	6	mM	67-20
Mg chlorination	1	1	1	1		
	0	0.5	1	2	(100 mM)	10x
	5	4.5	4	3	1120	

- enzyme added individually 0.25 μ L for 0.5 U }
0.5 μ L for 1 U }

To Page No. _____

Witnessed & Understood by me,

Date

Inv nt d by

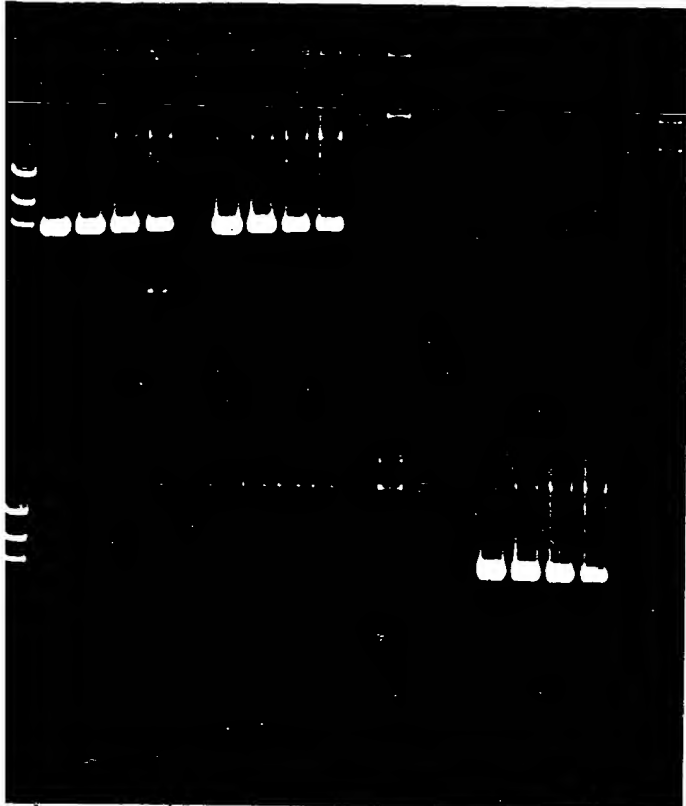
Dat

Record d by

K. Subramani

11/16/94

g N Regular 3'-1 PPT
0.5V



2 3 4 6
it 0.5 1
2 3 4 6 mol 12g
1V

du primer

Vent +/- exo didn't discriminate between modified and unmodified
" With du in earlier lines per 2 500 bp Lac 2 never got amplified

- samples divided 12/19/94

Result

- Regular - unmodified, revised, so couldn't say 3'-1 PPT is better than unmodified!
- du certainly has problem with Deep Vent.
- with the amount of product seen with 1V don't know why there is no product with 0.5V & 3'-1 PPT primers.

Can of template - ?
Centaminatin -
what are these bands on the top.

- Template / primer - no enzyme controls also have them?

To Page No. _____

ed & Understood by m ,

Date

11/28/94

Invented by

R corded by

K. Stachman

Date

11/18/94

UNIT ASSAY FOR T. mea Pol.

ag No. — 2/1/95 Purpose: DETERMINE THE UNIT CONCENTRATION OF TWO SAMPLES.

PREMIX:

✓ 335 μ l 2x REACTION BUFFER (1.5 mM TAPS, pH 9.3 / 50 mM KCl / 1 mM DTT)
✓ 1.3 μ l $HgCl_2$ @ 1.00 M
✓ 92.9 μ l DUA @ 3.5 μ g/ μ l
✓ 139.8 μ l H_2O
✓ 65 μ l H.C. @ 90.3 cpm/ μ mol

REACTION BUFFER:

I. 0.5 M Taps, pH 9.3 180 ml
FW = 243.3 g
TITRATED WITH 10 N NaOH 12.165 g

II. FOR 10 ml @ 2x

✓ 1 ml 0.5 M Taps, pH 9.3 = 50 mM
✓ 500 μ l 2 M KCl = 100 mM
✓ 40 μ l 0.5 M DTT = 2 mM

Assay @ 72°C, 10 min

	156	U/ μ l
1 μ l of 53 μ l @ Y40	65413	17.3
2 μ l	113154	15.0
3 μ l	151375	13.4
1 μ l of 5 μ l @ Y5	44554	1.48
2 μ l	95434	1.58
3 μ l	121699	1.35
1 μ l JH-61 @ Y100	19038	

$\bar{X} = 15.2$ U/ μ l
 $\bar{X} = 1.47$ U/ μ l

To Page No. _____

Ass d & Understood by me,

R. Plen

Dat

7/12/95

Invented by

Recorded by

Date

5/23/95

TNE

Page No. _____

12/95

Goal: To clone the TNE 35Fy (mut) into ptk99A.

PUC TNE 35Fy Clone #1	30	ptk99A	5
10x R4	5	10x R2	2
H2O	13	H2O	11
BspHI	2	NcoI	1
	50 μ l	H3	1

37°C - 1 hr.

Applied 5 μ l to 0.8% agarose gel. Gel run at 180V.

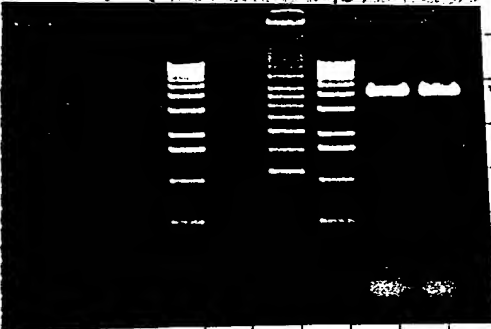
20 μ l

Applied to 0.8% agarose gel. Gel run at 180V.

Sl. uncut ptk99A/35Fy #1
BspHI

ptk99A/
NcoI/H3

7/14/95



cut out frag & freeze at 20°C

ptk99A/NcoI/H3 cut looks good
cut out 56 bp
4176
- 56
4120 bp

3' to 5' mut
pucTNE 35Fy #1 / BspHI gives 1kb, 1.3kb, + 8.7kb frag. Therefore, BspHI cuts pucTNE 35Fy #1 3X. There must be a BspHI in the insert.

13/95

ETOH ppt. Digest.
Dissolved in 20 μ l TE

BspHI

BspHI

5 μ l
5 μ l 1X BT
10 μ l

15 μ l DNA
2 μ l 1X R2
2 μ l H2O
1 μ l H3 10 μ l
20 μ l

Applied to 1 lane of 0.8% agarose gel. Gel run at 180V

37°C - 1 hr.

To Page No. _____

Issued & Understood by me,

Date

Invented by

Date

Lishu Xu

7/14/95

Recorded by

My Long

7/13/95

From Page No. _____

- Take pool from Hep. (cancer) and load into Q (cancer) syringe

BFAA.

(12)

2.5 mm Tris - 7.4 2.5 ml
 0.5 mm EDTA 1 ml
 5 mm Bml 3.58 ml
 10% glycerol 10 ml
 10 mM KCl 3.3 ml
 95-112 d H₂O (ms = 1.9)

- Take Bml from pool -

is in S. BFA 2.5 ml 1:5 w/ 500 ml 3500 ml A
 load. at 1 ml/min. (1.7 ms)

B = same (+) 1 M KCl. (6.7 ms)

1 - SA

2 - Bkg

3 - C

4 - CFA

5 - 10

6 - 15

7 - 18

8 - 20

9 - 22

10 - 24

11 - 24

12 - 28

13 - 30

14 - 32

15 - 34

61656.00

62.00

974.00

76.00

48.00

84.00

3696.00

1112.00

762.00

554.00

454.00

330.00

120.00

150.00

118.00

low SA - need new vial
 1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15.

1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15.

0.5 ml sayle - in 7.4 C / 1 ml

Total units

% Known

Blue load 3550

Blue pool 1500

Hep load 3550

Hep pool 5470

Q load 1248

Q pool 560

- (42%)

+ (154%)

(45%)

1514.00

2178.00

3058.00

2722.00

4326.00

4726.00

125066.00

125278.00

Test for Activity at 607 Fy (F607y). Check for pol. activity post H₂O kill.

- Disease per per go protocol

from Del's clone p. 124, Book 3573, #1 + color

- Spec Act = 78.3

- Coolers good! Activity post H₂O kill. Need Del's clone for p. To Page N

With ssed & Understood by me,

Date

Invented by

Date

R corded by

³²P primer for 14/ Vent
Human spleen DNA

Project N

Exhibit 2

Appl. No. 09/558,421

B k N

67

ge No. ³²P 2633 (into the anchor primer)
follow P. 53 except use more ³²P ATP

~26% primer
have ATP is
100% efficient
in labeling

| | | | | | | |
|---|--------------|---|---|---|----------------------|-----------------------------|
| go 2633 159 μ M | 1 μ l | ✓ | ✓ | ✓ | (159 μ M primer) | dry down |
| ³² P γ ATP 6000 Ci/mmol | 25 μ l | ✓ | ✓ | ✓ | (41.8 μ M ATP) | 1106 beads |
| 10 mCi/ μ l 10-21-94 | | | | | | 10 μ l H ₂ O |
| (11.67 μ M ATP) | | | | | | 1 μ l 34P DP |
| 5X Kinase buffer | 675 | ✓ | ✓ | ✓ | | 15' 37°C |
| PNK 50 μ l | 0.25 μ l | ✓ | | | | 1 μ l EDTA |
| | 33.75 | | | | | |

37°C 30 min \rightarrow 5' 55°C \rightarrow add

spin col same as P154, 7, and 145, 3

dilute ³²P 2633 with 100 μ l H₂O (V_f = 133 now)

spin in microfuge in "micron 3"

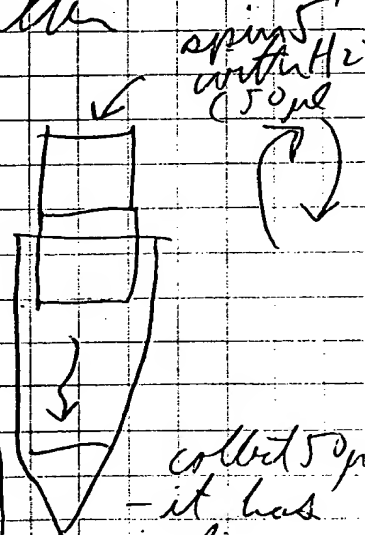
(micron # 42402) - after all venting, put

add 200 μ l more H₂O and spin again

remove volume that did not enter filter



invert filter



10-24-94

Had a problem: filter kept peeling back on micron 3. Maybe 9 fold was too high on Beckman microfuge "E" model will skip separation of free ATP.

³²P 2633 is diluted only 33.75 fold for $f = 4.71 \mu$ M

To Page No.

Read & Understood by me,

Steven Polak

Date

10/24/94

Inv. nted by

Recorded by

Date

10-19-94
10/24/94

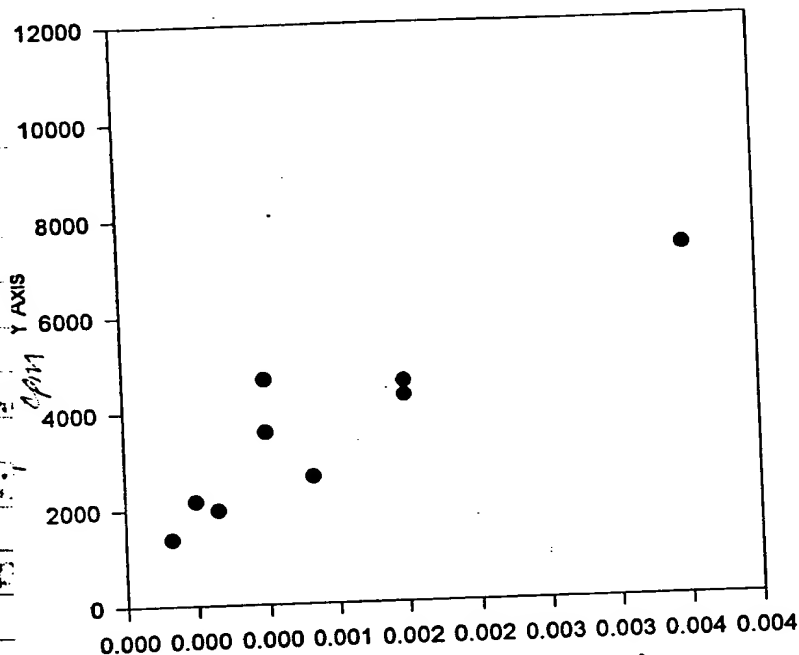
Project No. _____

Book No. _____

TITLE _____

04

From Page No. _____

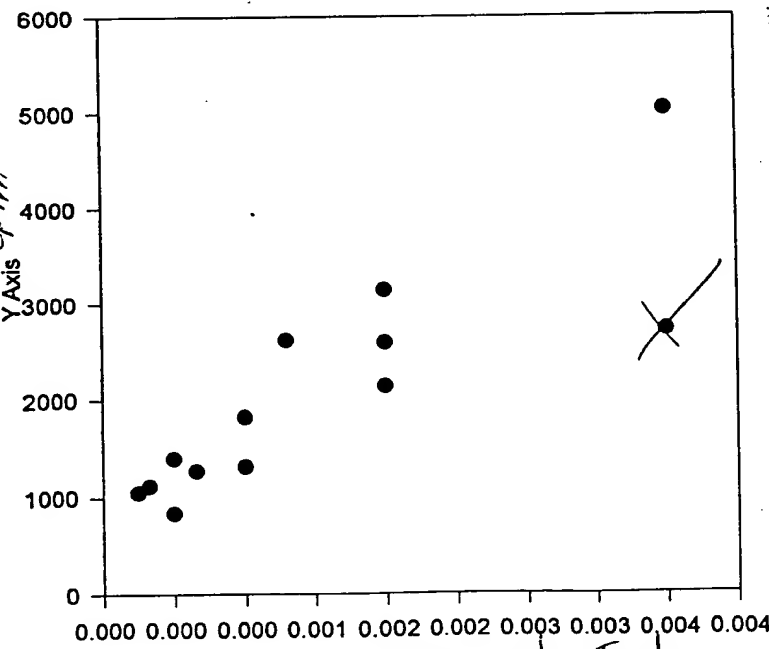


| | | |
|----|----------|-----------------------|
| 1 | 78.00 | |
| 2 | 1374.00 | 3.3×10^{-4} |
| 3 | 1962.00 | 6.6×10^{-4} |
| 4 | 2618.00 | 1.33×10^{-3} |
| 5 | 2154.00 | 5×10^{-4} |
| 6 | 3560.00 | 1×10^{-3} |
| 7 | 4262.00 | 2×10^{-3} |
| 8 | 4660.00 | 1×10^{-3} |
| 9 | 4556.00 | 2×10^{-3} |
| 10 | 7268.00 | |
| 11 | 91772.00 | |
| 12 | 92240.00 | $\bar{y} = 8.9450$ |
| 13 | 84328.00 | |

$8.9450(25) = 55.9 \text{ cpm/pmol} - \text{S.A.}$

$\text{Factor} = 1.61 \times 10^{-5}$

$(1.61 \times 10^{-5})(\text{cpm})(\text{DF}) = \text{U}/\mu\text{L}$



| 3AM | CPM1 | |
|-----|----------|----------------------|
| 1 | 160.00 | |
| 2 | 1052.00 | 2.5×10^{-4} |
| 3 | 828.00 | 5×10^{-4} |
| 4 | 1116.00 | 3.3×10^{-4} |
| 5 | 1262.00 | 6.6×10^{-4} |
| 6 | 2624.00 | 1.3×10^{-3} |
| 7 | 1392.00 | 5×10^{-4} |
| 8 | 1310.00 | 1×10^{-3} |
| 9 | 3140.00 | 2×10^{-3} |
| 10 | 1820.00 | 1×10^{-3} |
| 11 | 2134.00 | 2×10^{-3} |
| 12 | 5024.00 | 4×10^{-3} |
| 13 | 2592.00 | 2×10^{-3} |
| 14 | 2716.00 | 4×10^{-3} |
| 15 | 78604.00 | |

$\text{S.A.} \sim 48.7 \text{ cpm/pmol}$

$\text{Factor} =$

Witnessed & Understood by me, _____

Date _____

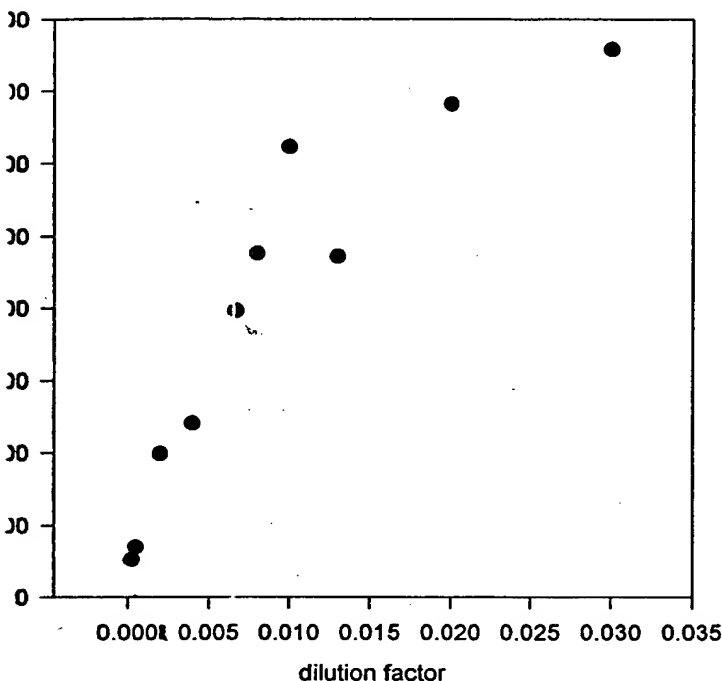
Invented by _____

Date _____

Recorded by _____

To Page _____

Mutant Taq Titration 12/13



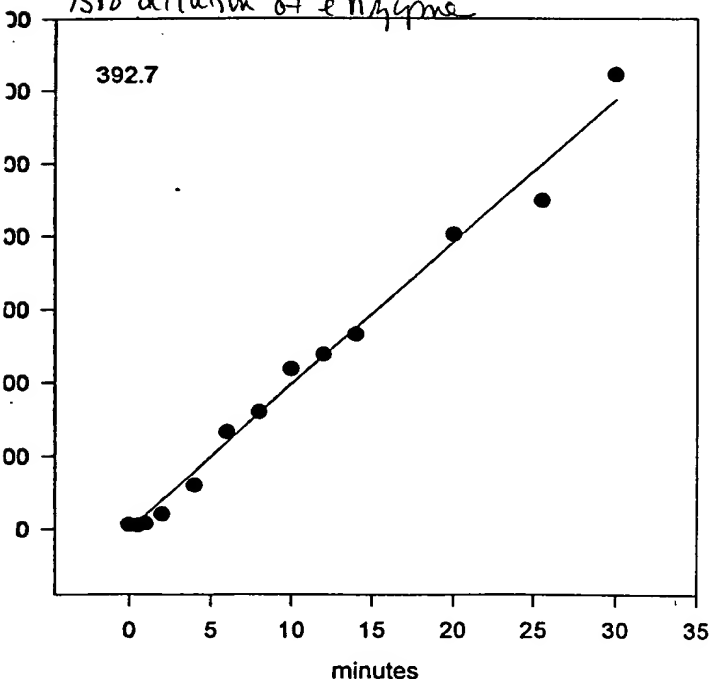
| 1 | 12470.00 | | |
|----|----------|------|------|
| 2 | 13642.00 | | |
| 3 | 15176.00 | | |
| 4 | 6342.00 | 33.9 | 32.9 |
| 5 | 7936.00 | 104 | 20.9 |
| 6 | 9428.00 | 1013 | 12.5 |
| 7 | 3970.00 | 33.0 | 33.0 |
| 8 | 4822.00 | 20.4 | 20.4 |
| 9 | 9530.00 | 21.1 | 21.1 |
| 10 | 3978.00 | 2 | 33.0 |
| 11 | 128.00 | | |
| 12 | 2624.00 | 1245 | 41.1 |
| 13 | 1364.00 | 178 | |
| 14 | 578.00 | | |
| 15 | 314.00 | | |
| 16 | 77492.00 | | |
| 17 | 76814.00 | | |
| 18 | 79502.00 | | |

$\bar{y} = 77936.48$

$$\text{Factor} = 1.85 \times 10^{-5}$$
 $\sim 25 \text{ V}/\mu\text{m}$

Time course analysis of mutant Taq - 12/14

1/510 dilution of enzyme



| SAM | CPM1 |
|-----|----------------------------|
| 1 | 100.00 ⁸ |
| 2 | 94.00 ³⁰ - Time |
| 3 | 160.00 1 |
| 4 | 396.00 2 |
| 5 | 1198.00 4 |
| 6 | 2630.00 6 |
| 7 | 3170.00 8 |
| 8 | 4340.00 10 |
| 9 | 4748.00 12 |
| 10 | 5322.00 14 |
| 11 | 8060.00 20 |
| 12 | 9000.00 25 |
| 13 | 12464.00 30 |

Keep nice - Add 10 μ l SM
EDTA to terminal
types

SDO μ l per ml
1.1 μ l dCTP 25
10 μ l KCO Tag-1

Heat $\approx 74^\circ\text{C}$

take out 25 μ L
aliquots at set
intervals into
fermentation
tubes -

Spot 20 μ l

GF/C filters
TCA wash

24 ml per mix } per exam
5 ml enzymes } time point

↓ 740C -

25 μ aliquots - 10 μ EDTA.

20

To Page No.

sed & Und rstood by me,

Date _____

Invented by

Date _____

Recorded by

111

4/15/91

Date 12/92

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

| SAM | CFM1 | U/ μ l |
|--------------------|----------|------------|
| 1 | 15576.00 | 54.5 |
| 2 $\frac{1}{500}$ | 27100.00 | 35.1 |
| 3 | 18258.00 | 57.3 |
| 4 $\frac{1}{1000}$ | 2950.00 | 34.5 |
| 5 | 3538.00 | |
| 6 | 43578.00 | |
| 7 | 84.00 | |
| 8 | 71702.00 | |
| 9 | 70582.00 | |
| 10 | 67698.00 | |

500 μ l mix + 1.1 μ l dGTP/L32-D48 μ l mix+ 1.2 μ l enzyme

10 min @ 24°C

quench w/ 10 μ l 5M EDTA + icespot 20 μ l on GF/C - TCA wash

SA - 43.7 cpm/pmol

$$\text{factor} = 2.0 \times 10^{-5}$$

$$(\text{cpm})(\text{factor})(\text{DF}) = \text{U}/\mu\text{l}$$

To Page No. _____

Witnessed & Understood by me, _____

Date

2/20/95

Invented by

Recorded by

Date

12/94

ge N .

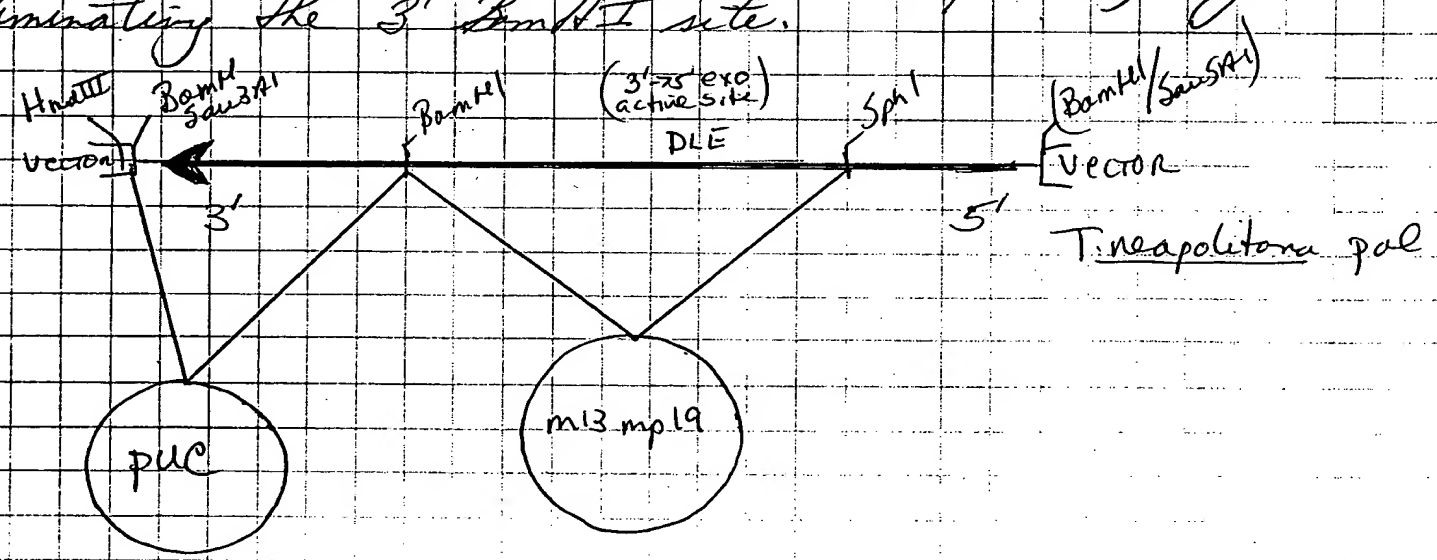
January 25, 1995 (Wednesday)

I'm BAAACK!!

after a tour of duty with Joel's group, a trip to Aulba and a few weeks vacation off I am back and ready to administer the fatal blow to this project. I will finish sequencing this gene, mutagenize it to conform to our needs, and overexpress it so people can enough enzyme to swim in it and still have money left over for a cup of coffee and a copy of the New York Times!

How's that for an opening!

First things first. Let's reclone the region of the *pal* gene we are interested in mutagenizing. Deb had I have had no success with the last clone. Secondly, let's make the subclone more user friendly by eliminating the 3' BamHI site.



strong BamHI/Sau3A
PCR
clone Hd3/BamHI

make ssDNA
D → A by SDM

To Page No. 52

| | | | |
|------------------------------------|----------------------------|----------------------------|-----------------|
| ed & Understood by me,
my forgo | Date
1/27/95
4/24/95 | Invented by
[Signature] | Date
1-25-95 |
| | Recorded by
[Signature] | | |

From Page No. 51

January 25, 1995 (Wednesday)

T. neapolitana / pSPORT DNA made by Michael Smith (not the Nobel Laureate; the horse boy)

DIGEST SCHEME

| | <i>T. nea</i> / pSPORT | mBap 19 | pUC 18 |
|--------------------|------------------------|--------------|--------------|
| (least 6) 10x B/R | 15 μ l ✓ | 13 μ l ✓ | 13 μ l ✓ |
| DNA | 2 ✓ | 2 ✓ | 2 ✓ |
| (10 μ l) BamHI | 1 ✓ | 3 ✓ | 3 ✓ |
| (10 μ l) SphI | 1 ✓ | 1 ✓ | 1 ✓ |
| Form | 20 μ l | 20 μ l | 20 μ l |
| (0.1 μ l) CAP | | | 1 μ l |

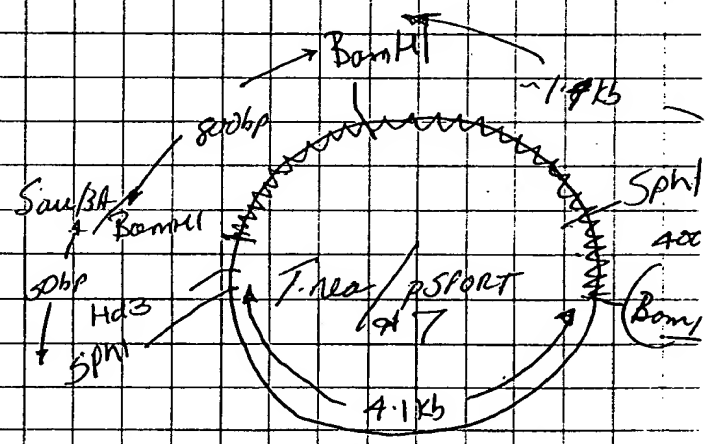
Incubated 37°C (heat-block) 1:00 → 2:45

0.8% Agarose Gel (1XTAE)
190 Volts

[Redacted]

[Redacted]

11/27/95



I forgot to run the 1Kb ladder. What a horse boy!

Fragment *T. nea* / pSPORT? BamHI / SphI show
be 4.5 Kb, 1.4 Kb, 0.8 Kb, 0.25 Kb.
Perhaps something partial. Try again
but do it separately.

Witness d & Understood by m ,

Dat

Invented by

Date

Man boy

11/27/95

R Corded by *[Signature]*

1-25-95

To Pag N

100

Project No. _____

Book No. _____

TITLE

F667K

From Page No. _____

Spin down 1.2 l cells - G-S-S - 7000 RPM - 30 min - Decant
- Dissolve 8.6 grams cells in 25 ml Crack BFR (pg 7)

concentrate 4 x 3.5 30 sec. (4) 6 at 4.5 30 sec each

A 540 1:200 diln

Crack - .98 \approx 70% crack. \rightarrow Heat 15 min @ 88°C.
Final - .13 cool 10 min / ice

ADD 0.4% PEG (1.2 ml 10% stock) - stir 15 min

Spin in 55-34 9K - 30 min - Decant sup

7.55 gms \rightarrow ADD NH_4SO_4 - 30 g / l (40% sat) - stir 4°C 45 min

Spin down in 55-34 1/2 hr 12K - Decant sup, save for activity
Resuspend pellet in 8 ml BFR A - dialyze 4 hr 4°C in 500 ml

BFR A

25 mM Tris - 7.4

8% glycerol

0.5 mM EDTA

10 mM KCl

5 mM Bmer (1.3 mM)

BFR B (high salt gradient)

Same (5) 2 mM KCl

TOSO-650 Heparin

Down Sul column Equilibrate w/ A

Load at 0.5 ml/min

wash w/ 8 VTs - until baseline at 1.5 ml/min

Change dialysis buffer once -

Bump 4 ml TOSO 650 Heparin - 4M Gu HCl - 3M NaCl 10 VTs
Wash with H_2O -

Equilibrate w/ BFR A - $\text{C}_{\text{RAD}} = (1.4 \text{ ms})$ $\text{C}_{\text{RAD}} = 7.1 \text{ ml} -$
load - 0.75 ml/min.

10 VT gradient A \rightarrow B - same position

Mix - premade by H.G. - stored @ -420°C - same rxn mix as for native

With ss d & Understood by me,

Date

2/27/85

Inv nted by

[Signature]

Dat

12-5200

R c rd d by

T Page

Page N

purpose: Amplification of PMC 9 with different amounts of Tag - titration.

Deep vent
Tag + Deep vent

all samples discarded

Constant different con - titration

using dV primers, 1 μ M

200 μ M dV

1 μ M primer dV 2728 + 2729 (100 μ M)

100 μ g template diluted 100 ng / λ \rightarrow 1 ng / λ

Deep vent buffer

Titration a. Tag: 0, 0.5, 1, 1.5, 2, 2.5 and 5 U

b. Tag: Deep Vent: 1:0, 1:0.001, 1:0.005, 1:0.01, 1:0.05
1:0.1, 1:0.2, 1:0.5, 1:1, 1:2

c. Deep Vent 0, 0.025, 0.05, 0.1, 0.5, 1, 2, 5

prepared a master mix w/o enzyme tubes added separately

Did just one of each.

H₂O 116.7

10 x buffer 150

45 μ l + 5 μ l (enzyme + H₂O)

primer 1 15

2 15

Tag: 5 U / λ diluted to 1 U / λ in 1x buffer. Template 3 μ l

Enzyme H₂O

0 = - 5 D.V 2 U / λ \rightarrow 1 U / λ \rightarrow 0.01 U / λ 45 μ l

0.5 = 0.5 4.5

0.001 U / λ

1 = 1 4

0.025

2.5

0.01

1.5 = 1.5 3.5

0.05

5

8 - 15

2 = 2 3

0.1

1

0.1

2.5 = 2.5 2.5

0.5

5

5 = 5.0 -

1.0

0.5

2 U / λ

2.0

4

5.0 = 2.5 μ l

To Page No.

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Date

Inv nted by

Date

Recorded by

11/18/94

K. Strehman

From Page No. _____

| T | D.V | Tag | W/x | D.V | detected in | Time |
|--------|-------|-----|-----|-----|-------------|------|
| | | | | | 1x super | |
| Unit 1 | 0 | 1 | μl | | | 16 |
| | 0.001 | | | 1 | | 17 |
| | 0.005 | | | 5 | | 18 |
| | 0.01 | | | 1 | | 19 |
| | 0.05 | | | 5 | | 20 |
| | 0.1 | | | 1 | | 21 |
| | 0.5 | | | 5 | | 22 |
| | 1.0 | | | 15 | | 23 |
| | 2.0 | | | 1 | | 24 |

- Thermocycled at 94°, 5'

30 (94°, 30", 56°, 45 sec, 72°, 3')

- after 6 cycles noticed dNTP was not added (1) & 20 added 1 μl of 10 mM dNTP (200 μM / 500 μM 2X) individually each tube & started again the cycling!

maybe have to repeat again.

Tag alone Deep vent alone

Tag: Deep vent



No Contamination: X
(no enzyme)

.001 .005 .01 .05 .1 .5 1 2

- so much mispriming.

Repeat & in duplicate, use Lambda / Hind III marker

Witnessed & Understood by me,

Date

Invented by

Date

To Page 1

TNE

age No. _____

12/95

Goal: To clone the TNE 35FY (mut) into pTrc99A.

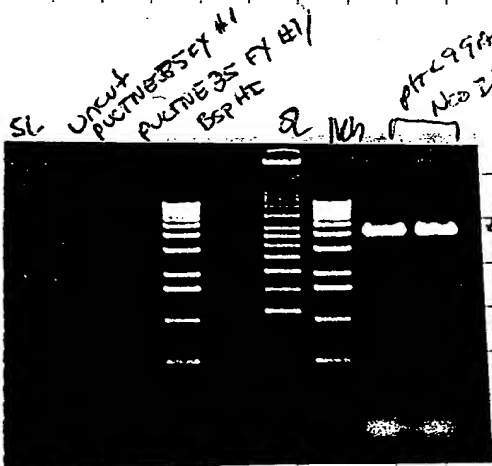
| | | | |
|-----------------------|----------|------------------|----|
| PUC TNE 35FY Clone #1 | 30 | pTrc99A | 5 |
| 10xR4 | 5 | 10xR2 | 2 |
| H ₂ O | 13 | H ₂ O | 11 |
| BspHI | 2 | NcoI | 1 |
| | 50 μ | H3 | 1 |

37°C - 1 hr.

Applied 5 μ l to -
0.8% agarose gel
Gel run at 180V

20 μ l

Applied to
6.0% agarose gel
Gel run at 180V.



cut out frag & ligate at 20°C

pTrc99A / NcoI / H3 cut looks good
cut out 56 bp
4174
- 56
4120 bp

pUC TNE 35FY #1 / BspHI gives 1 kb, 1.3 kb, + 2.7 kb frag. Therefore, BspHI cuts pUC TNE 35FY #1 3x. There must be a BspHI in the insert.

13/95

EtOH ppt. Digest.
Dissolved in 20 μ l TE

BspHI
5 μ l
5 μ l 1x8.3
10 μ l
Applied to 1 lane
of 0.8% agarose gel
Gel run at 180V

BspHI
15 μ l DNA
2 μ l 1xR2
2 μ l H₂O
1 μ l H3 100:1
20 μ l
37°C - 1 hr.

To Page No. _____

Issued & Understood by me,

Date

Invented by

Date

L. Zhou Xu

7/14/95

Recorded by

My Long

7/13/95

³²P primer for 14/1 Vent
Human spleen DNA

Project N ._____

Book No._____

age N — 32P 2633 (into the anchor primer)
follow P. 53 except use more 32P ATP

~26% primers have ATP in
^{100%} efficiency in calculation

(1) (159 pm primer)

1 µl ✓ ✓ ✓
25 µl ✓ ✓ ✓

(2) (41.8 pm ATP)

1 µl H₂O
1 µl 34P dTP

15' 37°C
1 µl EDTA

nigro 2633 159 µM
32P γ ATP 6000 Ci/mmol
10 mCi / ml 10-21-94
(1.67 µm ATP)
5x Kinase buffer
PNK 50 u/ml

675
0.25 µl
33.75

37°C zu 55°C \rightarrow add

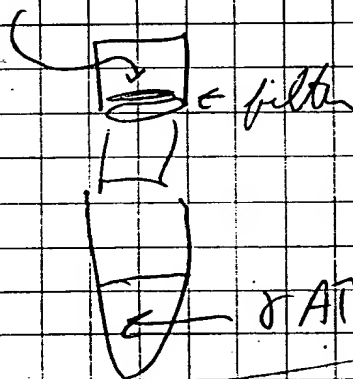
~~spin col same as P154, 7, and 145, 3~~

dilute ³P2633, with 100 μ l H₂O ($V_f = 133$ now)

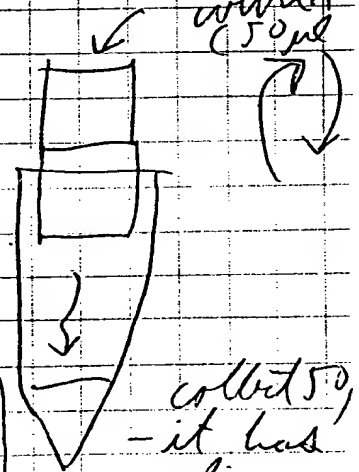
spin in metaphase is "micron 5"

spin in microfuge in "micron S"
(amicon # 4240?) - after all went in, put
add 200 μ l more H_2O and spin again

d. remove volume that did not enter filter



invert filter



Had a problem: filter kept peeling back on micron 3. Maybe g force was too high on Beckman microfuge "E" model will skip separation of free ATP.

^{32}P 2633 is diluted only 33.75 fold for
CF = 4.71 μM

To Pag No.____

essed & Understood by me,

Teacher's Policy

Date

10/24/94

Invent d by

Recorded by

Date _____

10-19-84
10/24/84

108

Project No. _____

Book No. _____

TITLE 100gram Crack Tne

From Pag No. _____

Growth: 764 D1 00 IR -100g

Slurry cells in 200ml of crack buffer - lg: 2ml
Final volume - 300ml -

Crack buffer -
25mM Tris pH 7.4
1mM EDTA
1mM PMSF
8% glycerol
5mM Bme

Filter cells slurry through 4 layers
of cheese cloth

Pass through gauze (mini) 2x @ 100

Set up 90°C heat bath before cracking cells. use -
floor shaker -

Innoculate @ 85°C for 12 minutes - with light shaking -
cool immediately on ice water bath ~15 min -

Spin @ 18,000 xg in GSA rotor - collect supernatant -
- 40 min - bright yellow color -

PEI precipitation - 4% PEI + 50mM KCl final concentration
add slowly over 20 minutes
let stir an additional 45 min - spin down @ 18,000 xg
in GSA rotor - 40 minutes -

MS (NH₄)₂SO₄ precipitation -

Add (NH₄)₂SO₄ solid to a final of 60% saturation -
Add slowly over 30 minutes - let stir o/n @ 4°C

100mL : $\frac{x}{250}$

To Pag N

Witnessed & Understood by me,

Date

Invented by

Dat

May Lopez

4/5/95

Rec rd d by

E. Flynn

03/29/95

T. neapolitana SDM

Tag No. 52

January 26, 1995 (Thursday)

DIGEST SCHEME:

| | | <i>T. nea</i> /pSPORT | | m13 mp19 (~270ng/ul) | |
|---------------------|-------|-----------------------|---|----------------------|---|
| 1 (React 3) | HOH | 24.5 μ l | ✓ | 22.5 μ l | ✓ |
| | DNA | 3 | ✓ | 3 | ✓ |
| | DNA | 1 | ✓ | 3 | ✓ |
| F-107 (104/ μ) | BamHI | 1.5 | ✓ | 1.5 | ✓ |
| | Total | 30 μ l | | 30 μ l | |

Incubated 37°C (heat block) 8:04 → 9:08

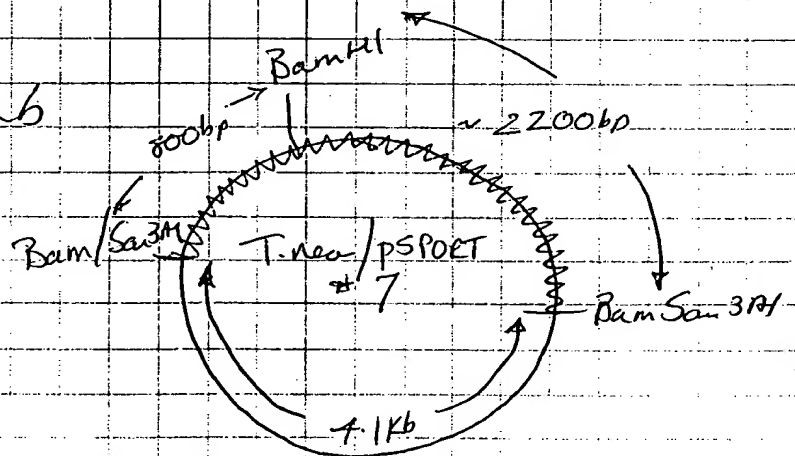
✓ 3 μ l removed for analytical gel.

| | | <i>T. nea</i> /pSPORT | mp19 |
|---------------|------------|-----------------------|------|
| DIGEST | 27 μ l | ✓ | ✓ |
| IMKCE | 2 | ✓ | ✓ |
| HOH | 9 | ✓ | ✓ |
| 1/ μ SphI | 2 | ✓ | ✓ |
| Total | 40 μ l | | |

Incubated 37°C (heat block)
 9:17 → 10:25

Agarose Gel (1XTAE)
 190V/16

Comment



To Page No. _____

Used & Understood by me,

Date

Invented by

Date

Mer Loryo

1/27/95

Recorded by

Drumf. Schmidt

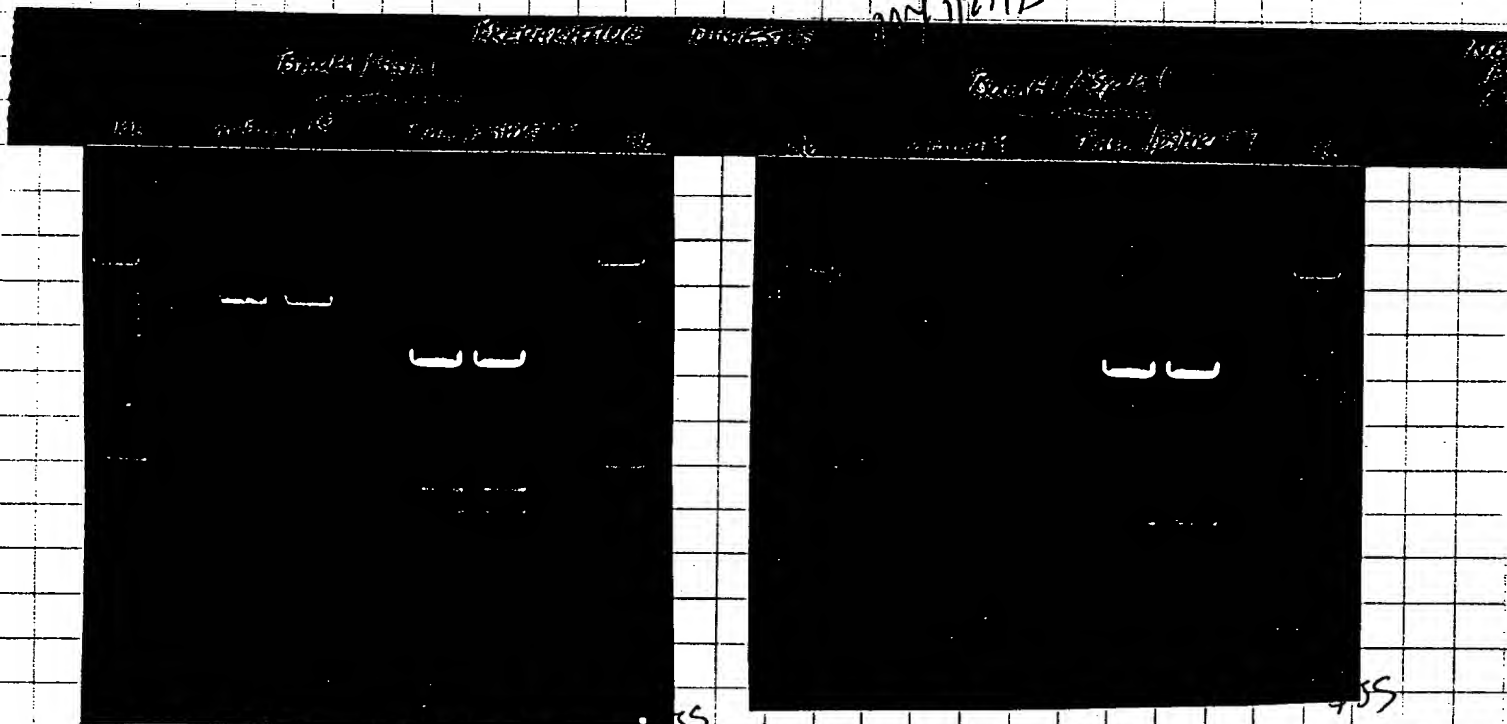
1-26-95

From Page No. 53

January 26, 1995 (Th)

0.8% Agarose Gel (1xTAE); Run at 190 Volts

JAN 27 1995



Bands extracted from the gel and placed in the same tube. The DNA was purified away from the agarose using Gene Clean as described by the manufacturer (BIO-101).

DNA eluted in 14 μ l H₂O

LIGATION SCHEME

| | | | |
|---------------------|-----|------------|---|
| ETB 402 (Ligase) | DNA | 14 μ l | ✓ |
| 5X Bfr | | 4 | ✓ |
| 14 μ l (Ligase) | | 2 | ✓ |
| Form | | 20 μ l | |

Incubated 22°C (room-temp)
2:15 → 3:15

→ 1 μ l ligation / 2 μ l for transformation

Witnessed & Understood by me,

Date

Initiated by

Date

M. J. Long

1/27/95

Recorded by

D. J. K. K. K.

1-26-95

Trineapolitona 50M

Pr j ct No. _____

B ok No. _____

55

ag N 54

January 26, 1995 (Thursday)

DH10B Electrocompetent

20 μ l DH10B Electrocompetent Cells + 1 μ l (of a 1/3 dilution; Serp 54)

2.5 KV

1ml LB, 37°C air shaker 20 min

→ 10% applied to LB plate in 4ml Soft Agar (0.7%)
90% + IPTG (1mM) and X-gal 100 μ l of 4%

incubated 37°C incubator

1/27/95

To Page No. _____

Used & Understood by m ,

Date

Invented by

Date

May Longo

1/27/95

Recorded by

Dr. J. Schmidt

1-26-95

Page No. _____

Topic: pMC9 amplification, using 40 primers # 2722 & 2729

by titration: buffers / KlenTaq
 Deepvent

w/ 100 dNTP

100 primer each

2 pg template pMC9 / Act II

100 mM Mg

cycling:
 20 (94° 30", 56° 30", 72° 30")

prepared 15 x w/o enzyme → added separately in 1x buffer

| x buffer (DV) | 75 | (KT) 75 | 5 | 50 / x | 1 ml |
|------------------|-------|---------|-----|--------|------|
| dNTP | 15 | 15 | 2.5 | | 1.5 |
| primer 1 | 7.5 | 7.5 | 2 | | 2 |
| " 2 | 7.5 | 7.5 | 1.5 | | 1.5 |
| Mg | — | 15.0 | 1 | 10 / x | 1 |
| template | 3.0 | 3.0 | 1.5 | | 1.5 |
| H ₂ O | 642.0 | 627.0 | 0 | | 0 |

distributed 50 µl / tube added enzyme.

| 2 | 1 | 2 | (tube #) | 16 | 17 |
|---|----|----|----------|----|----|
| — | 3 | 4 | | 18 | 19 |
| | 5 | 6 | | 20 | 21 |
| 5 | 7 | 8 | | 22 | 23 |
| 1 | 9 | 10 | | 24 | 25 |
| 5 | 11 | 12 | | 26 | 27 |
| — | 13 | 14 | | 28 | 29 |

0.01 15 30
 Deepvent mix

↑
 rep Vent buffer KlenTaq buffer

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Date

Invented by

Date

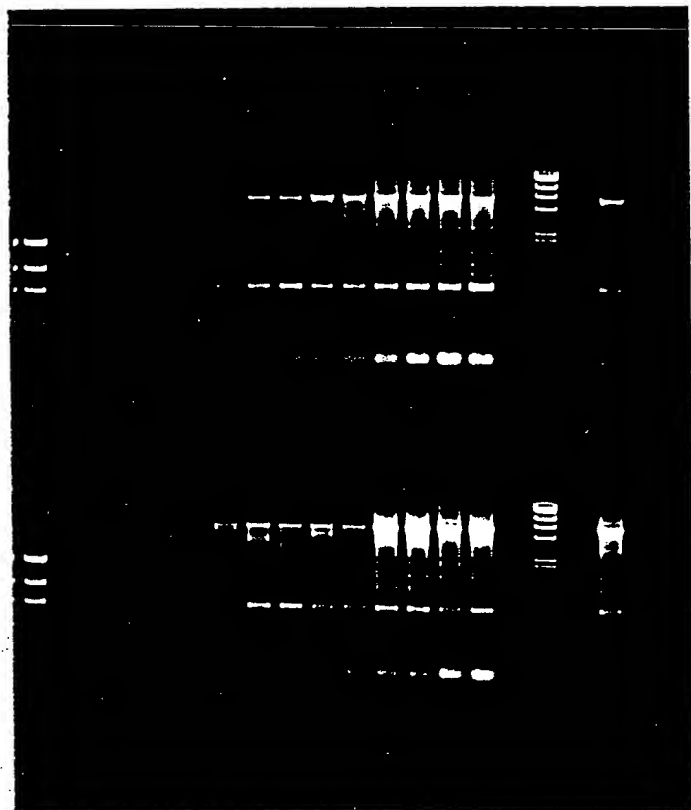
Record d by

11/22/94

K. Silberman

From Pag No. _____

0 .5 1 1.5 2 2.5 5



0 .5 1 1.5 2 2.5 5 1:0.01 max

Tag like above.

← D.V. buffer

← K.T. buffer

Result: more product with increasing amounts of Tag expected.

- K.T. B / 1U better than D.V. / 1U
- 1:0.01 better than 1U Tag alone.
- K.T. B more product than D.V. buffer
- But lot of mispriming - adjust the cycling conditions

T Pag N

Witnessed & Understood by me,

Date

Invented by

Dat

Record d by

11/22/94

K. Subramaniam

TNE

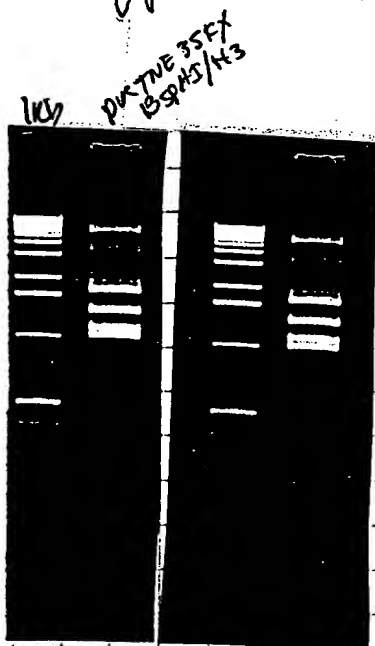
Page N. _____

4/95

7/17/95
DNY

puc TNE 35EX / BspH1E → ETOH ppt. → Dissolved in 200 μ l 1x,
butler. 2 μ l of H3 (100:1) was added. 37°C - 1h.
applied to 1 lane of a 1% LMP agarose gel.
Gel run at 180V.

1x
7/20/95



cut the 200bp frag out &
ligate at -20°C.

← 200bp frag

95

used the ~~phenol~~ phenol extraction method to purify DNA.
Dissolved in 10 μ l TE.

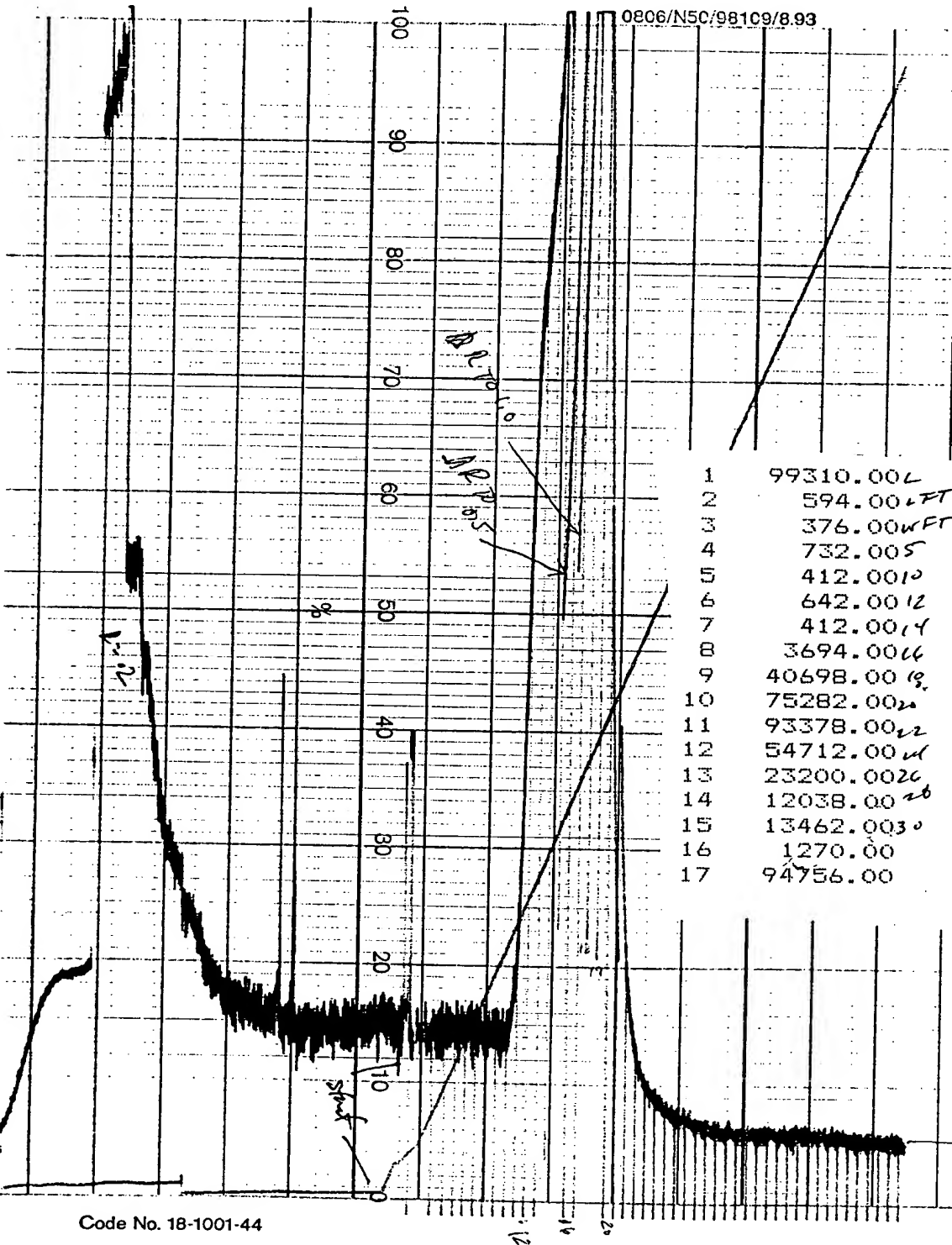
To Page No. _____

| | | | |
|--|-----------------|--------------------------|-----------------|
| Read & Understood by me,

Lizhu Yu | Date
7/20/95 | Invented by
Ming Long | Date
7/18/95 |
| | Recorded by | | |

TOSO - Hg 650(m)

sg N .



| | |
|----|------------|
| 1 | 99310.00L |
| 2 | 594.00LFT |
| 3 | 376.00WFT |
| 4 | 732.005 |
| 5 | 412.0010 |
| 6 | 642.0012 |
| 7 | 412.0014 |
| 8 | 3694.0016 |
| 9 | 40698.0018 |
| 10 | 75282.0020 |
| 11 | 93378.0022 |
| 12 | 54712.0024 |
| 13 | 23200.0026 |
| 14 | 12038.0028 |
| 15 | 13462.0030 |
| 16 | 1270.00 |
| 17 | 94756.00 |

74°C - 8 min
 24 WFT mix
 1 ml sample - STOPW/ 1000 EPTA
 X-CST - STOPW/ 1000 EPTA
 1 L 5
 2 LFT
 3 WFT
 4 5
 5 10
 6 12
 7 14
 8 16
 9 18
 10 20
 11 22
 12 24
 13 26
 14 28
 15 30
 16 32
 17 34
 18 36
 19 38
 20 40
 21 42
 22 44
 23 46
 24 48
 25 50
 26 52
 27 54
 28 56
 29 58
 30 60
 31 62
 32 64
 33 66
 34 68
 35 70
 36 72
 37 74
 38 76
 39 78
 40 80
 41 82
 42 84
 43 86
 44 88
 45 90
 46 92
 47 94
 48 96
 49 98
 50 100

Pool - 1779
 - Daily CVS
 BFR - A -

Code No. 18-1001-44

To Page No. _____

Read & Understood by me,

Date

Invented by

Date

Recorded by

105

2/27/96

2-10-96

Human spleen DNA

Project N _____

Exhibit 4

Appl. No. 09/558,421

B k No. _____

67

age N _____ ^{32}P 2633 (into the anchor primer)
 follow P. 53 except use more ^{32}P ATP

| | | | | | | | | | | | | |
|--------------------|--------------|--------------|---|----------------------|-------------|------------------------------|--------------------|----------|----------------|-------------|-----------|---|
| iso 2633 | 159 μM | 1 μl | ✓ | (159 μM primer) | 2633 | 159 μM | 1 μl | ✓ | 2633 | 159 μM | 1 μl | ✓ |
| ^{32}P 8 ATP | 6000 Ci/mmol | 25 μl | ✓ | (41.8 μM ATP) | 1106 ladder | 100 μl H ₂ O | 1 μl 34P dGTP | 15' 37°C | 1 μl EDTA | | | |
| 1000 Ci/ μl | 10-21-94 | 675 | ✓ | ✓ | | | | | | | | |
| (1.67 μM ATP) | | 0.25 μl | ✓ | | | | | | | | | |
| 5x Kinase buffer | | 33.75 | | | | | | | | | | |
| PNK 50 μl | | | | | | | | | | | | |

37°C 30 min → 5' 55°C → add

spin col same as P. 54, 7, and 145, 3

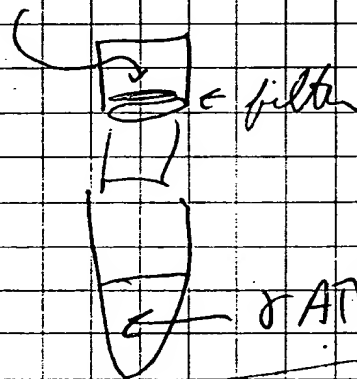
dilute ^{32}P 2633 with 100 μl H₂O ($V_p = 133$ now)

spin in microfuge in "micron 3"

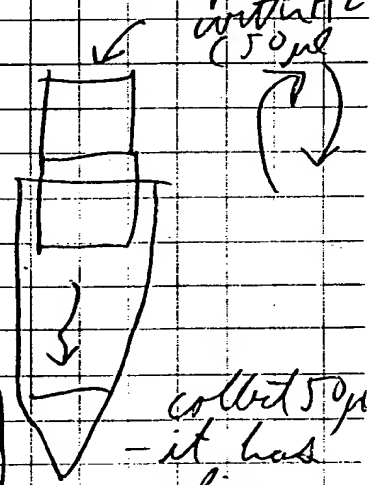
(micron # 4240?) - after all went in, put

add 200 μl more H₂O and spin again

remove volume that did not enter filter



invert filter



10-24-94

Had a problem: filter kept peeling back on micron 3. Maybe g force was too high on Beckman microfuge "E" model will skip separation of free ATP.

^{32}P 2633 is diluted only 33.75 fold for $C = 4.71 \mu M$

To Page No. _____

as d & Und rstood by me,

Steven A. Pokany

Date

10/24/94

Invented by

Recorded by

Date

10-19-94
10/24/94

Project No. _____

Book No. _____

TITLE 13.5 Kb long PCR

From Page No. _____

 ^{32}P 2633 4.71 μM ① ✓ 4.7 μl ② ✓ 4.7 μl 0.2 μl 2628 old 199
dilute to 10 μM ✓ 2.2 μl →0.2 μl 80 ng/ μl Human
spleen DNA✓ 1.1 μl →(80 ng/ μl)

4 dNTPs 10 mM each

✓ 2.2 μl →200 μM

Pol max

Vent 2 μl 0.52 μl Tf1 1 μl 15 μl Vp = 15.5 μl →✓ 1.36 μl

4.08

Total
1.32 unit
0.087 μl Vent
in ①. 3
more in ② =
0.28 μl Vent

5X buffer
Cheng

✓ 2.2 →

Mg (OAc)₂
12 mM

✓ 1.1 →

Cp = 1.2

H₂O

32.2/1.2 =

65.44

75.34

Vp = 110 μl

62.7

72.6

110 μl

remove 10 μl to 2 μl 0.2 M EDTA at 0 cycles.
remove 10 μl at 5, 10, 15, 20, 25, 30, 36

program

139

15", 94°C

→ 20 min, 68°C

140

10", 72°C

141

1", 94°C

142 = 141, 139, 140, 4

started at 8:16 AM
20.5 min/cycle

need 12 hr, 20 min to
complete so expect to end
at 8:40 - 9:00 PM

T Page No.

Witnessed & Understood by me,

Deena Golap

Date

10/24/94

Invent d by

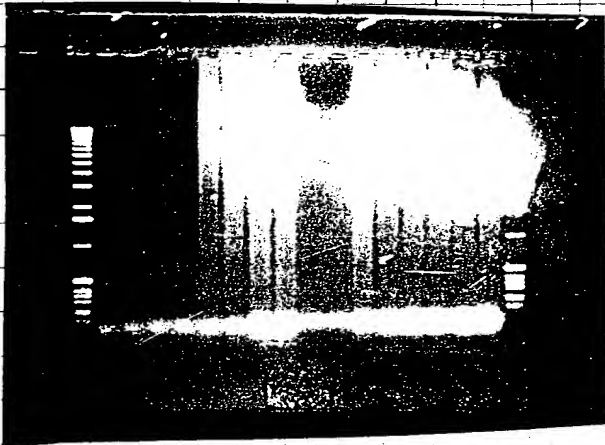
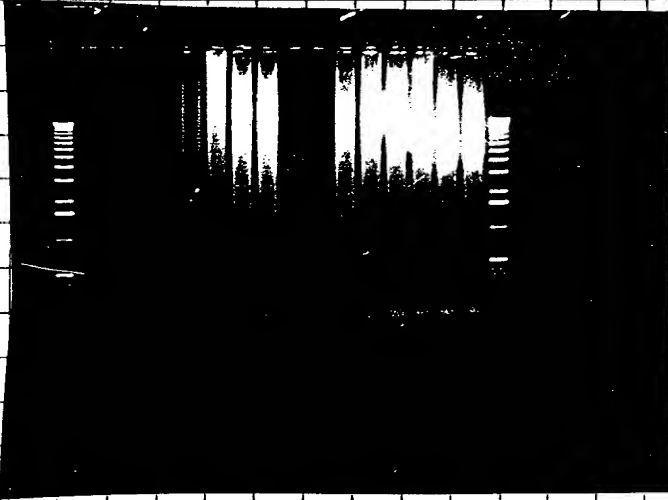
Record d by

Date

10-24-94

Fig N. — 8 1/2 square same as P. 56

If) : 1.33 4
 05 10 15 20 25 30 36 05 10 15 20 25 30 36



To Pag No. _____

ed & Understood by me,

Date

Invent d by

Dat

Richard Bokrup

10/24/94

Recorded by

Project No. _____
 Block No. _____

The over Heparin 40mL column.

Tag No. _____

Spin $(\text{NH}_4)_2\text{SO}_4$ sol'n - @ 18,000 x g 40 minutes -
 in GSA rotor -

Save supernatant -
 Save pellets -

Store one pellet in -20°C - process the other
 pellet 2

2: pellet 1 slightly greater than half - $\sim 3/5$

pellet 2 slightly less than half - $\sim 2/5$

Resuspend pellet in 20 mL of Buffer 1 -

Buffer 1

5 mM Tris pH 7.5
 3.1 glycerol
 40 mM KCl
 5 mM Bme
 .1 mM PMSF

dialyze - against Buffer 1 for ~ 8 hrs -
 Exchange buffer 4 times -

heparin column - use prepacked Heparin from A.G. -
 ~ 40 mL column - bump w/ Buffer + KCl -
 wash w/ H_2O -

Previously A.G. used 3 mL Heparin a
 5 gram crack

Direct scale up = $\frac{3}{5} = \frac{4}{5} = 30$ mL Heparin
 ~ 50 g

equilibrate w/ Buffer 1 \rightarrow (Note: made 20 mM KCl -)

To Page No. _____

| | | | |
|---------------------------------------|----------------|----------------------|------------------|
| Used & Understood by me,
May forso | Date
4/5/95 | Invented by E. Hyman | Date
03/30/95 |
| | | Recorded by | |

From Page No. _____

Conductivity of Load - 2.8 mS - after ~8 hrs of dialysis

Notice a small precipitate matter in dialysis tube -
Spin down in SS-34 - 18,000 x g - 10 minutes -
same pellet - small + white -

① Load - 21 mL of sample - 7.5 ~~mm~~ mL/min - collect FT -

② Wash - 2 V_t of Buffer 1 - collect ^{7.5} 8 mL fractions
1 mL/min

③ Gradient - Buffer 1 to Buffer 2 - 25 mM Tris pH 7.5
8% glycerol
5 mM BME
1 mM PMSF
2 M KCl

10 V_t - 400 mL gradient - linear - 1 mL/min -
collect 7.5 mL fractions -

④ Wash w/ 2 V_t Buffer 2 1 mL/min - 7.5 mL fractions -

Let column run O/N -

Note: Next time gradient should be much shallower - 1 M KCl -

To Page N

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

-Mary Long-

4/5/95

03/31/95

T. neapolitana SDM

Project N 2022

B ok No. 3884

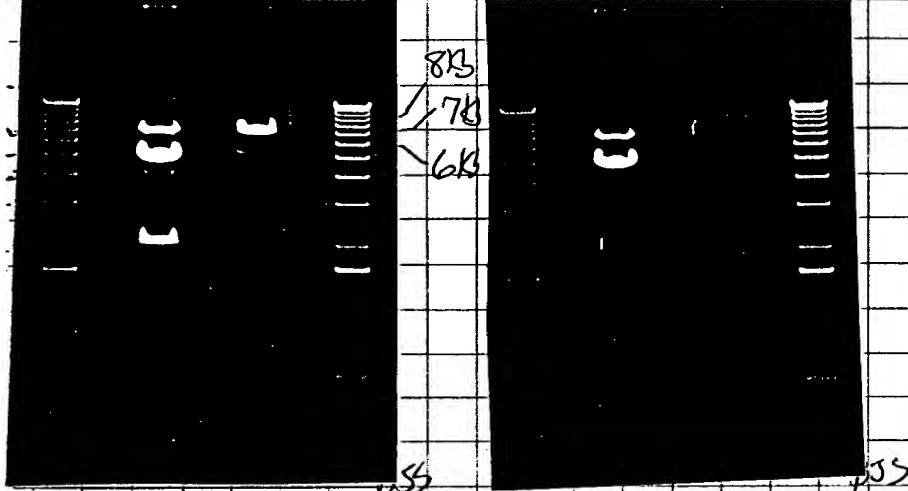
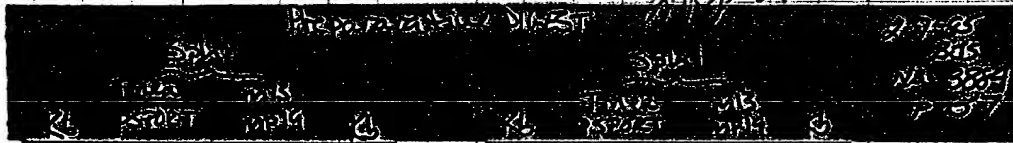
57

N 56

February 7, 1995 (Tuesday)

0.8% Agarose Gel (1X TAE); 75 V 16

2/16/95



Bands extracted from the gel and the DNA purified away from the agarose using Gene Clean as described by BIO-101.

DNA eluted in 14 μ l HOH

LIGATION SCHEME

| | | | |
|----------------|----|---------|---|
| HOH | - | μ l | |
| 5X Bfr | 4 | | ✓ |
| DNA | 14 | | ✓ |
| WAT DNA Ligate | 2 | | ✓ |
| Total | 20 | μ l | |

Incubated 3:23 pm \rightarrow 4:03 at room-temperature ($\sim 22^{\circ}\text{C}$)

3 μ l removed for transformation

152 F' IQ Competent All Transformation
0.1 μ l competent cells + 3 μ l ligation (see above)
2 min on ice, 35 seconds at 42°C water bath
1. and 90 l. applied to LB + No Antibiotic plates in
4 ml 0.7% Top Agar + 100 μ l 2% X-Gal + 10 μ l 100 mM IPTG
incubated 16 hours at 37°C incubator

To Page No. 58

d & Understood by me,

Date

Invented by

Dat

Neeraj Soni

2/16/95

Recorded by

Neeraj Soni

2-7-95

11/21/94

PMCP / Tag + D.V.

Page No. _____

Tag 1 U + Deep Vent different amount in PCRing
PMCP.

Deep Vent buffer 22x KlenTag buffer.

| | | | |
|------------------|-------|-------|-----------------|
| 10x buffer | 110 | 110 | 200 µl dNTP |
| dNTP | 22 | 22 | 1 µl primer |
| Mg | — | 22 | 200 µg Template |
| primer 1 | 11 | 11 | 1 µg 200 µl |
| 2 | 11 | 11 | |
| Template | 4.4 | 4.4 | |
| H ₂ O | 895.6 | 873.6 | |

added 1 U Tag in 1 µl in 1x buffer.

added different amount of Deep Vent in 2 µl in 1x buffer
either D.V. or RT buff.

* Tubes.

| Tag | Deep Vent | Deep Vent buffer | K. T. buffer |
|-----|-----------|------------------|--------------|
| 0 | 0 | 1 2 | 22 23 |
| 1 | 0 | 3 4 | 24 25 |
| | .001 | 5 6 | 26 27 |
| | .005 | 7 8 | 28 29 |
| | .01 | 9 10 | 30 31 |
| | .05 | 11 12 | 32 33 |
| | .1 | 13 14 | 34 35 |
| | .5 | 15 16 | 36 37 |
| | 1 | 17 18 | 38 39 |
| | 2 | 19 20 | 40 41 |

94°, 3' 21' 42'
94°, 30", 56°, 30", 72°, 3' 1 : 0.01 mix

checked the cycling file instead of stop file - 2 cycles down
before changing to stop file. To Page No. _____

Seen & Understood by me,

Date

Invented by

Date

Recorded by

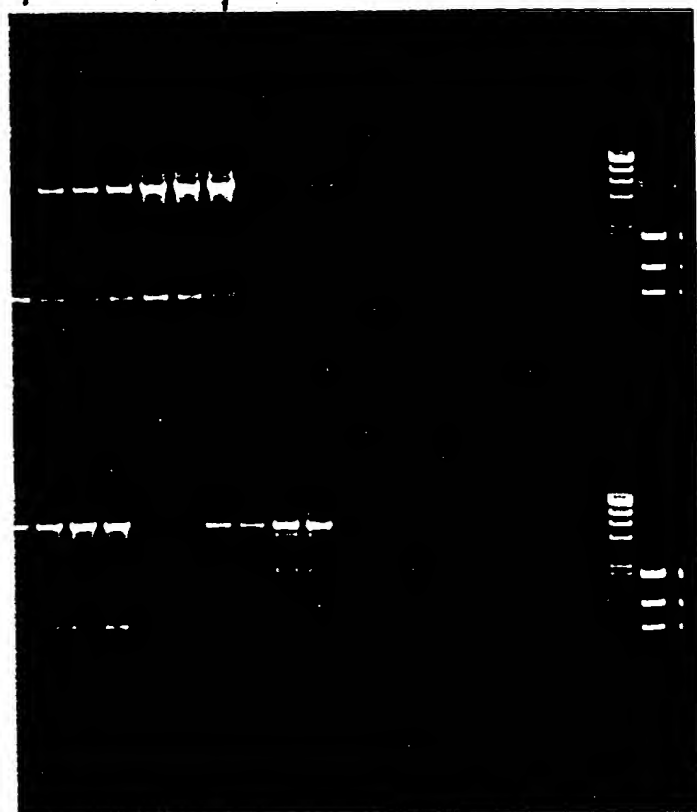
11/22/94

J. S. Srinivasan

Project No. _____

Book No. _____ TITLE _____

From Page No. _____



1 1:00.01 1:01 1:01 —————> 1:02

1:00.05 1:05

?

- maybe didn't add any Tag - make primer to next
- mispriming still there - check annealing temp.
- Try again with new D. vent.
- Increasing D.V. over 0.05 = having D.V. alone. Tag effort nil.

discarded

12/19/94

Tag: Depvent
libation

← D.V. Buffer - cpl6
1:00.01 ok

← R.T. Buffer can go
cpl6 1:00.05 but
this can't D.V. more
mispriming

To Page N

Witnessed & Understood by me,

Dat

11/28/94

Invented by

Recorded by

K. Sturman ..

Dat

11/22/94

TNE

Page N _____

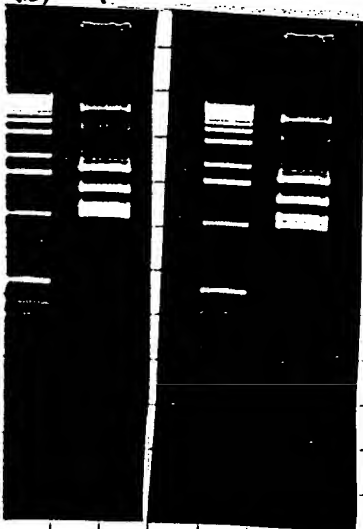
1/95

7/14/95
GNY

pucTNE 35FX / BspH I → ETOH ppt → Dissolved in 20ul 1x1
buffer. 2ul of H3 (100/1) was added 37°C - 1hr.
applied to 1 lane of a 1% LMP agarose gel.
Gel run at 180V.

1X
7/20/95

cut the 200bp frag out &
ligate at -20°C.



← 200bp frag

95

Used the ^{GNY 7/17/95} phenol extraction method to purify DNA.
Dissolved in 10ul TE.

To Page No. _____

Read & Understood by me,

Date

Invented by

Date

Litu Xu

7/20/95

Recorded by

Thuy Long

7/18/95

Project No. _____
Book No. _____

TITLE SDS gel Thermostable pols

72

From Page No. _____

| | mw | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| Tfl epicenter
lot TFS0809A
5 μ /ml | 1000 | | | | | | | | ✓ |
| Tfl MBR 5 μ /ml
cat # 111202 (Tfl)
lot 40104 | 100 | | | | | | | | ✓ |
| Tth MBR 5 μ /ml
lot 21021, cat 1115-02 | 100 | | | | | | | | |
| RTth Perkin Elmer, 2.5 μ /ml
cat N808-0007
lot 9189 | 170 | | | | | | | | |
| sequaltherm epicenter 5 μ /ml
lot 0140303 | 100 | | | | | | | | |
| Vent (NEB) 2 μ /ml
lot 17, assayed 7/94 | 250 | | | | | | | | |
| Deep Vent (NEB) 2 μ /ml
lot 4, assayed 8/94 | 250 | | | | | | | | |
| RTag EKBTI 40 μ /ml
H ₂ O | 1.25 μ l | | | | | | | | |
| TCA 15%
(see P 50-7 for TCA ppt) | 300 μ l
V _f = 100 μ l | | | | | | | | |
| <p>30' ice 10' microfuge at 4°C, remove supernatant
 vortex pellet in ice cold acetone, microfuge 10', remove supernatant
 dry 37°C 25', resuspend in 60 μl 1x cracking buffer</p> | | | | | | | | | |

Witnessed & Understood by me,
Deena Polarp

Date
11/29/94

Invent d by
Record d by

Date
10-25-94

T Page No.

g No. —

| | 9 | 10 | 11 |
|----|-----|---------------|-----|
| MU | TNR | Native
Tog | Tnc |
| | | | MW |

remains as per P148, 6

Start 29 mA at 1:35 PM
gel as per P140, 6

To Page No. —

To Page No._____

Read & Understood by me,

Dat

Inv nted by

Date

Ernst Polenz

11/29/94

Recorded by

10-25-84

Pag N _____

purpose: To amplify pMC9 with Deep Vent alone
 tried in Deep Vent buffer only.

| | |
|------------------|-----|
| 10x buffer | 100 |
| dNTP | 20 |
| Mg | — |
| primer 1 | 10 |
| primer 2 | 10 |
| Template | 4 |
| H ₂ O | 846 |

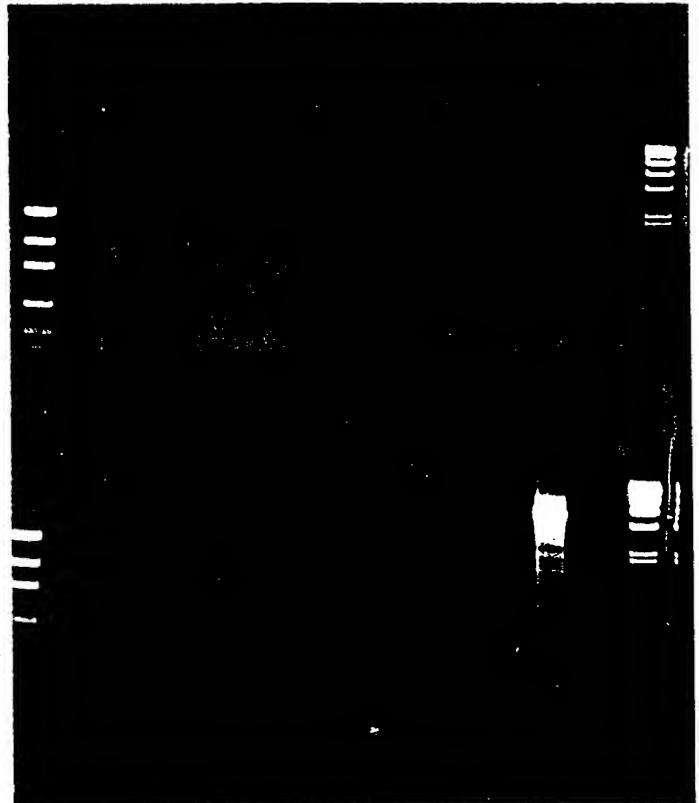
| | |
|--------|----------|
| 200 µl | dNTP |
| 2 mM | Mg |
| 1 µl | primer |
| 200 µl | Template |

94° 30'

30(94°, 30', 56°, 30', 72°, 30',

| | | |
|------|----|----|
| 0 | 1 | 2 |
| .001 | 3 | 4 |
| .005 | 5 | 6 |
| .01 | 7 | 8 |
| .05 | 9 | 10 |
| .1 | 11 | 12 |
| .5 | 13 | 14 |
| 1 | 15 | 16 |
| 2 | 17 | 18 |

result: Once again
 rep. Vent alone by itself
 didn't amplify anything
 with this dv primers.
 See the other set to
 confirm.



To Page No. _____

Issued & Understood by me,

Date

Invented by

Date

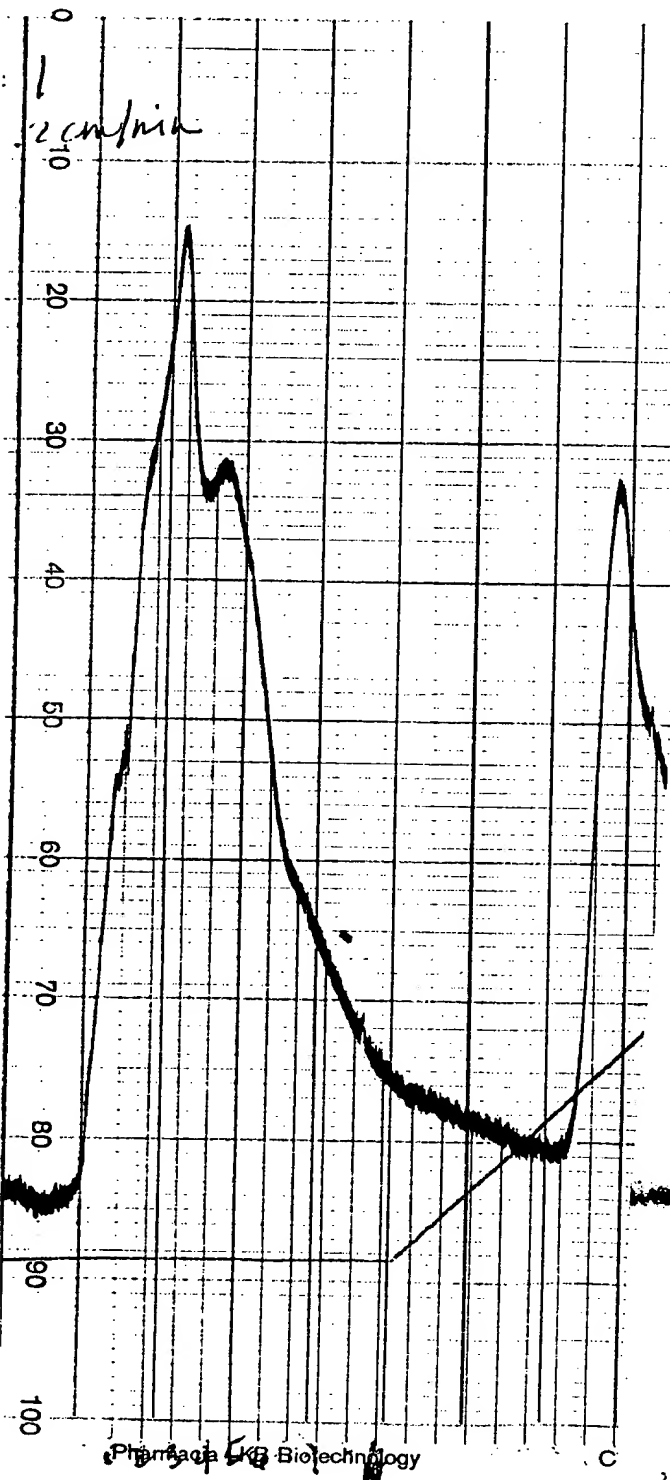
Recorded by

11/22/94

A. Sitarman

40 ml Heparin

ag No. _____



3/31/95

nm
4/5/95

To Page No. _____

| | | | |
|---|--------------------|----------------------------------|----------------------|
| Read & Understood by me,

May Longo | Date

4/5/95 | Inv. nted by
<i>E. Algren</i> | Date

05/31/95 |
| | | Recorded by | |

From Page N ____

SAM CPM1

| | | |
|----|-----------|------|
| 1 | 135612.00 | lead |
| 2 | 310.00 | 1 |
| 3 | 460.00 | 2 |
| 4 | 512.00 | 3 |
| 5 | 386.00 | 4 |
| 6 | 308.00 | 5 |
| 7 | 1118.00 | 6 |
| 8 | 960.00 | 10 |
| 9 | 546.00 | 15 |
| 10 | 420.00 | 16 |
| 11 | 1368.00 | 17 |
| 12 | 6588.00 | 18 |
| 13 | 45516.00 | 19 |
| 14 | 70278.00 | 20 |
| 15 | 98796.00 | 21 |
| 16 | 91534.00 | 22 |
| 17 | 109058.00 | 23 |
| 18 | 129224.00 | 24 |
| 19 | 73534.00 | 25 |
| 20 | 32032.00 | 26 |
| 21 | 13662.00 | 27 |
| 22 | 3166.00 | 28 |
| 23 | 2848.00 | 29 |
| 24 | 1910.00 | 30 |
| 25 | 1508.00 | 31 |
| 26 | 1426.00 | 32 |
| 27 | 3168.00 | 33 |
| 28 | 1278.00 | 34 |
| 29 | 840.00 | 35 |
| 30 | 516.00 | 36 |
| 31 | 119806.00 | |
| 32 | 121684.00 | |
| 33 | 123400.00 | |
| 34 | 26.00 | |
| 35 | 44.00 | |
| 36 | 50.00 | |

POOL

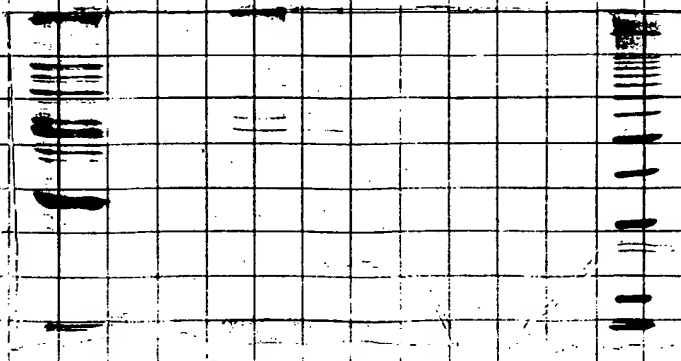
Add 22 μ l of LdCTP to 1ml of premix -
(From A.G.)

aliquot 24 μ l to pre-labeled ependants
 add .5 μ l of sample - incubate 10 min @
 72°C quench on ice + add 10 μ l of 5MED
 Spot 20 μ l on GF/C filters -
 wash w/ 10% TCA + 1% PPi - x1
 1% TCA x4
 EtOH x2

dry + count

Pool dialyzed O/N (sat). 104/01/95

Gel of Nucleic Acid Fractioning



11/1/95

11/1/95

Witnessed & Understood by me,

Date

Invented by

Date

To Page N

Mary Jones

4/5/95

Recorded by

E. Ryan

03/31/95

7 column Bradfads → 10 Y. PAGE

Project No. _____

B ok No. _____

113

ag N _____

Conc (mg/ml)

| | |
|-----------|-----------|
| 11.274956 | Cude |
| 0.307192 | Heat Kill |
| 1.065601 | PEI |
| 0.819939 | AS sup |
| 3.081949 | A Load |
| 0.385722 | 17 |
| 0.375194 | 18 |
| 0.285862 | 19 |
| 0.329252 | 20 |
| 0.368813 | 21 |
| 0.432621 | 22 |
| 0.980098 | 23 |
| 0.798244 | 24 |
| 0.419222 | 25 |
| 0.177069 | 26 |
| 0.069870 | 27 |

- too Not a. lide enough

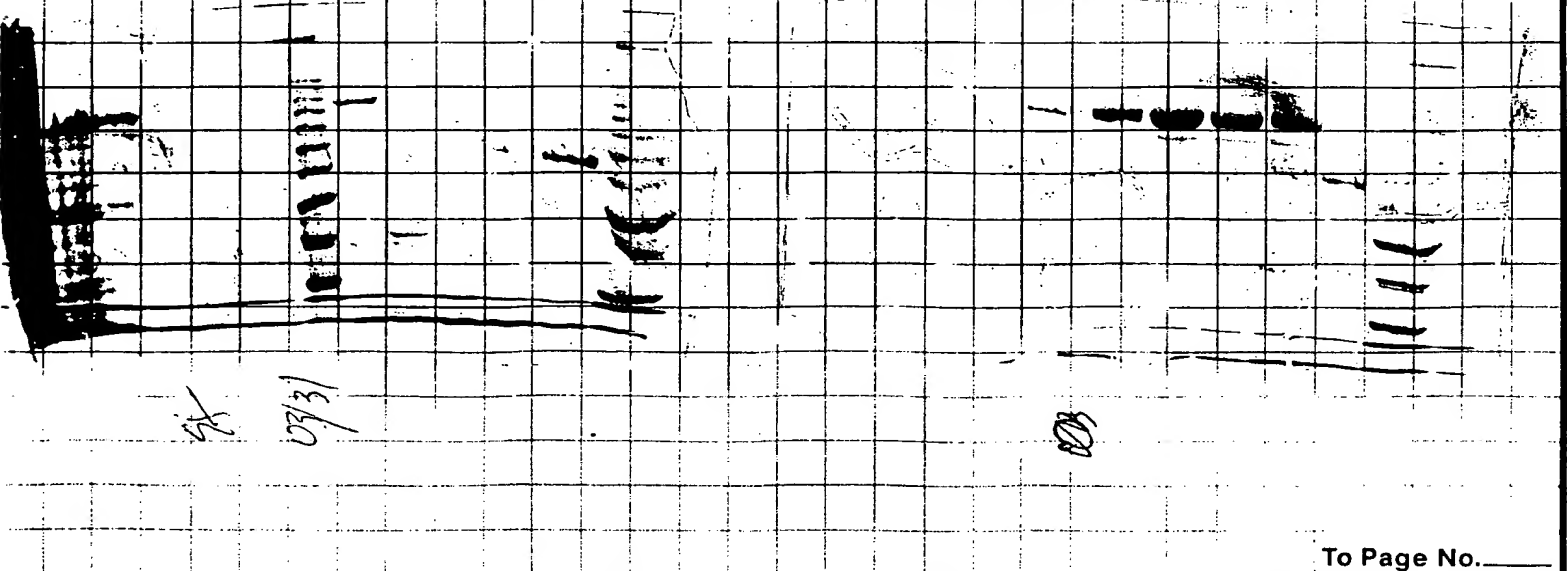
27 03/31

1. PAGE -

| | | | | | | | | | |
|-----|------|-----|------|---|------|----|----|----|----|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| ude | Heat | PEI | AS | M | Load | 17 | 18 | 19 | 20 |
| | Kill | | Sup. | | | | | | M |

| | | | | | | | | | |
|----|----|----|----|----|----|----|----|----|-----|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | EXR |
| 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | M |

03/31/95



To Page No. _____

| | | | |
|---------------------------------------|----------------|-----------------------|------------------|
| sed & Understood by m ,
Mary Forre | Dat
4/15/95 | Invented by
E. Kym | Date
03/31/95 |
| | | Recorded by | |

TNE

Page N. _____

Goal: To clone the TNE 35 Fy (mut) into pTTQ19 or a similar vector.

New Scheme: pUCTNE 35 Fy (≈ 5.1 kb) → H3 → Klenow → SphI → SclI/purified 2 kb (ScaI)

Clone into the SmaI/SphI site of pTTQ19.

| | | |
|------------------|----|-------|
| pTTQ19 | 4 | ≈ 2.5 |
| 10xR2 | 4 | |
| H ₂ O | 30 | |
| SmaI | 2 | |
| 100 μl | 40 | |

30°C - 1 hr
8/3/95

| | | |
|------------------|-------|-------|
| pUCTNE 35 Fy | 20 | ≈ 1.5 |
| 10xR2 | 4 | |
| H ₂ O | 14 | |
| H3 | 2 | |
| 100 μl | 40 μl | |

8/3/95 (M)

- 1 pUCTNE 35 Fy cut with H3
- 2 pTTQ19 cut with SmaI

Cuts look good

| | |
|--------------|----|
| pTTQ19/SmaI | 40 |
| SphI | 2 |
| 100 μl | 42 |
| 37°C - 1 hr. | |

| | |
|-----------------|-------|
| pUCTNE 35 Fy/H3 | 40.1 |
| 10xR2 | 10.1 |
| 10 mM dNTP mix | 2.1 |
| Klenow | 0.5.1 |
| | 52.5 |

ice 5'
EDTA to 20 mM
phenol extract
TA 100 μl

Read & Understood by me,

iburk

Date

8/3/95

Invented by

Recorded by

May Longo

Date

7/31/95

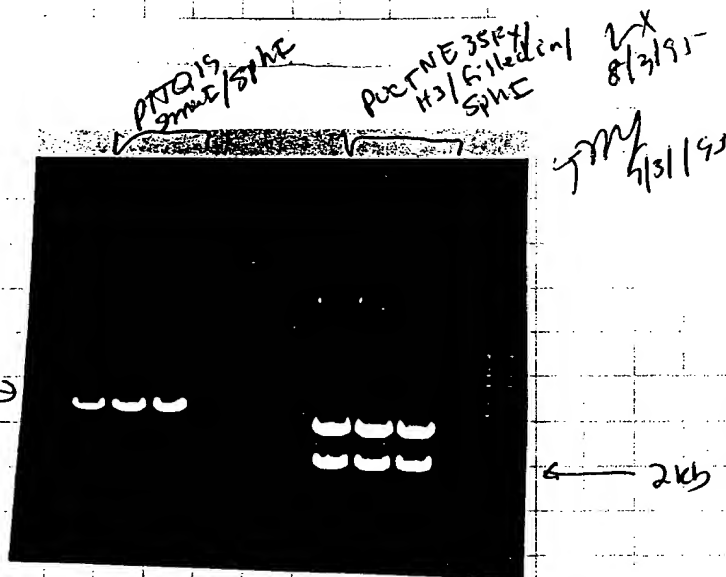
Project No. _____

Book No. _____

TITLE TNE

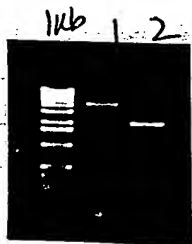
From Page No. _____

pUCTNE35Fy/H3 / filled in → resuspended in 40 μ l 1X R6
 2 μ l of 100 μ l SphI
 37° C - 1 hr.
 applied to a 0.9% agarose gel.
 Gel run at 180V.



cut bands out +
 freeze at -20°C.

GeneClean the frag as usual.
 Dissolved in 10 μ l TE.
 Applied 1 μ l to a 0.9% agarose gel.
 Gel run at 180V.



- 1 pTA15 / smc2 / sphI
- 2 2 kb H3 / filled in / sphI frag
 from pUCTNE35Fy

~ 10 ng/ μ l = .1
 ~ 20 ng/ μ l = .1

To Page N

With ss d & Understood by me,

Lidunyan

Date

8/8/95

Invent d by

Recorded by

Lidunyan

Date

8/1/95

TNE

Project N _____

B k No. _____

183

ag N _____

lig

TQ19 / SmaI / SphI .003 pmol/.1
 16 H3 / Filled in / SphI .015 pmol/.1
 5X ligase buffer
 H₂O
 Ligase (10)

| |
|------|
| 2 |
| 1.5 |
| 1 |
| 4 |
| 12.5 |
| 1 |
| 20.1 |

RT - 30 min.

Jason xformed 2ul of the lig with 100ul DH10B CC.
 std xform. Plated 10% + 90% on yet amp plates. 37°C ON

#2 10% 90%
 18 ~150

picked 8 colonies into 3 mls of CG + amp^r 100. 37°C - ON.

mp as usual. Dissolved in 5ul TE.

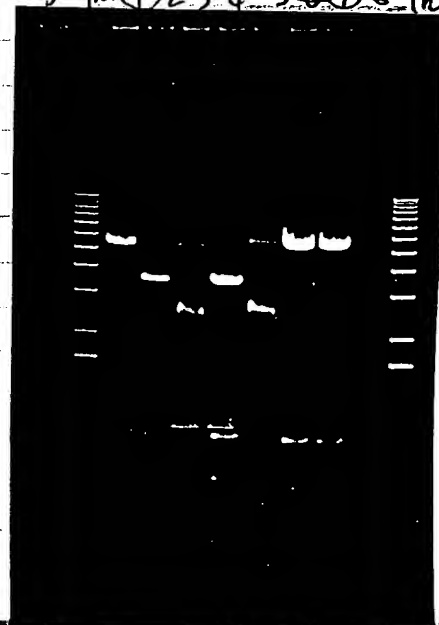
mp 3
 IDRL 2
 H₂O 13
 SphI 1
 EcoRI 1
 10

37°C - 1hr.

Applied to a
 0.5% agarose
 gel. Gel
 run at 100V

sub PUCTNE 35 PY mot into
 SmaI / SphI site of pTQ19
 clones cut E SphI / EcoRI
 5 kb 1 2 3 4 5 6 7 8 9 10

ANY 8B/55



To Page No. _____

sed & Understood by m ,

Date

Invented by

Date

Lisha Xu

8/3/95

R corded by

CONY Longo

2/3/95

58

Project No. 20221

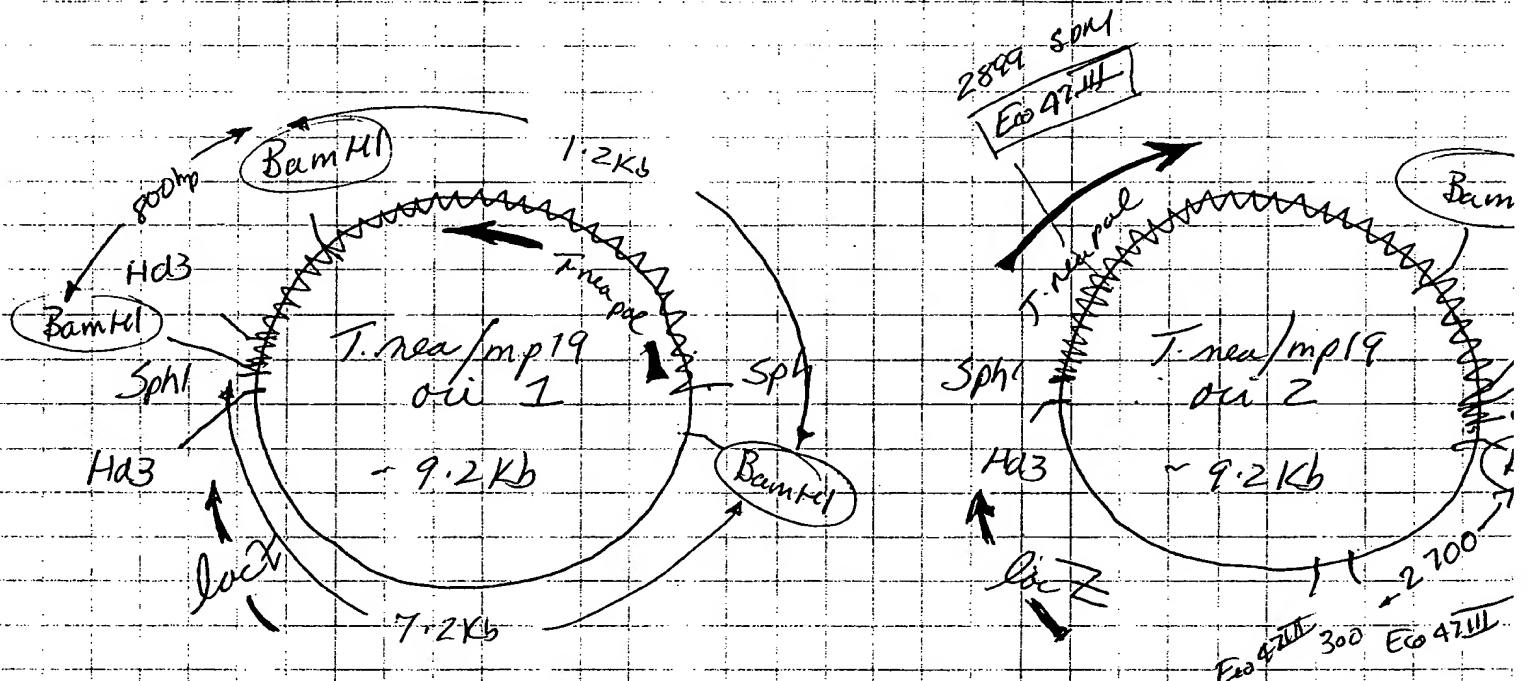
Book No. 3884

TITLE *T. neapolitana* SDM

From Page No. 57

February 8, 1985

- I added 200 μ l of DH5 α F' IQ lawn cells to 10 ml. circle brown.
- I added 1 ml of the cells to 8 glass tubes
- Each tube was inoculated with a clear plug and incubated at 37°C (8:00 am →)



| | | |
|-------|--------|----------|
| SphI | 7.2 Kb | VECTOR |
| | 2 Kb | INSERT |
| BamHI | 7.2 Kb | VECTOR |
| | 1.2 Kb | } INSERT |
| | 0.8 Kb | |

| | |
|--------|------------|
| 7.2 Kb | VECTOR |
| 2 Kb | INSERT |
| 8.4 Kb | VECTOR + I |
| 0.8 Kb | INSERT |

Add *T. nea*/pSPORT as a positive control for both digests

Witnessed & Understood by me,

May Longo

Date

2/14/85

Invent d by

Recorded by

Robert Schmidt

Date

2-8-85

To Page

ag No 58

February 8, 1995 (Wednesday)

DIRECT SCHEMES

| | PER RXN | x 9 = | COCKTAIL | |
|--------------------|----------|-------|----------|-------------------------------------|
| 1. HOH | 7 μ | x 9 = | 63 μ | <input checked="" type="checkbox"/> |
| React 6) 10x Bfr | 2 | x 9 = | 18 μ | <input checked="" type="checkbox"/> |
| DNA | 10 | | | |
| (100 μ) Sph I | 1 | x 9 = | 9 μ | <input checked="" type="checkbox"/> |
| Totm | 20 μ | | 90 μ | |

For T-neap/SPORT
control add

Tp E, 20 μ ☒
 DNA 2 μ ☒
 Totm 22 μ ☒

| | PER RXN | x 9 = | COCKTAIL | |
|---------------------|----------|-------|----------|-------------------------------------|
| HOH | 7 μ | x 9 = | 63 μ | <input checked="" type="checkbox"/> |
| React 3) 10x Bfr | 2 | x 9 = | 18 | <input checked="" type="checkbox"/> |
| DNA | 10 | | | |
| (100 μ) Bam HI | 1 | x 9 = | 9 | <input checked="" type="checkbox"/> |
| Totm | 20 μ | | 90 μ | |

add 10 μ to reaction

Continued on page 1 of Notebook 3966

Aroni - Patel

Gel photo

T Page No. _____

sed & Und rstood by me,

May Longo

Date

2/11/95

Invented by

Recorded by

Diana J. Lander

Date

2-8-95

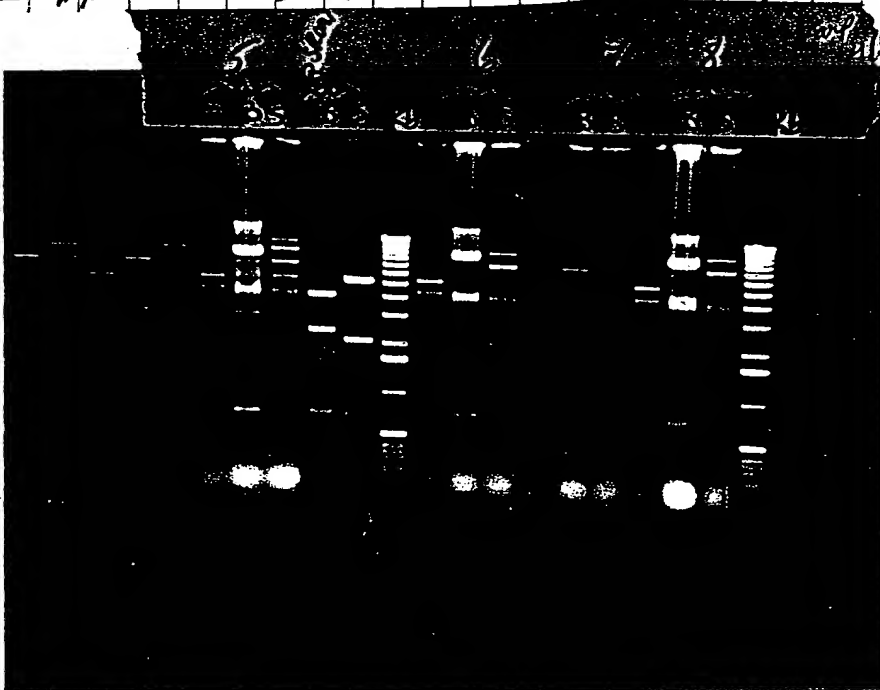
ig N _____

Con'd from 3884 NB

2/18/95 wed

MINIPREP DNA

- cfg 500 μ l of cells for 1 minute in an eppendorf cfg (centrifuge)
- removed supernatant and resuspended pellet in 100 μ l of 1X PEBI (SI) ^(saved)
- added 200 μ l of alkaline - SDS mix
- placed the tubes on ice for few minutes (3-5 min)
- added 150 μ l of 7.5 M Ammonium Acetate
- Mixed the tubes by inverting
- cfg the tubes for \sim 7-10 min.
- transferred 400 μ l supernatant to the new eppendorf tube
- added 800 μ l of ethanol to supernatant. Mixed tubes.
- incubated the tubes for \sim 2 min. Spin.
- dissolved pellet in 50 μ l of TE + RNase A.
- applied 5 μ l to a 1% agarose gel.



SI = 0.9% glucose

25 mM Tris HCl (pH 8.00)

10 mM EDTA

alkaline - SDS mix = 1% SDS

0.1 N NaOH

To Page No. _____

Used & Understood by me,

Date

Invented by

Date

Recorded by

Dwan

4/12/95

Book No._____ TITLE

TITLE

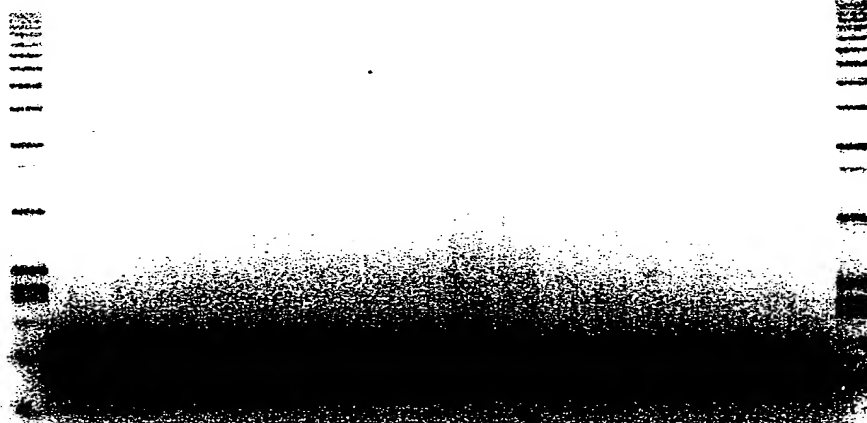
TF (units)

1.33

4

Agarori

10 5 10 15 20 25 30 36 | 0 5 10 15 20 25 30 36 cycles



O/N exposure 100 - 150,000 grains

Result: no 13.5 Kb product - maybe too much Mg^{+} with hot primer!

nonspecific screen ~~see~~ seen with E+Br (P69) is
cold in auto rad ~~above~~ above

To Page N

Witnessed & Understood by me,

Date

Invented by

Date

Deena Golap

" 29/11/4

Recorded by

10-27-94

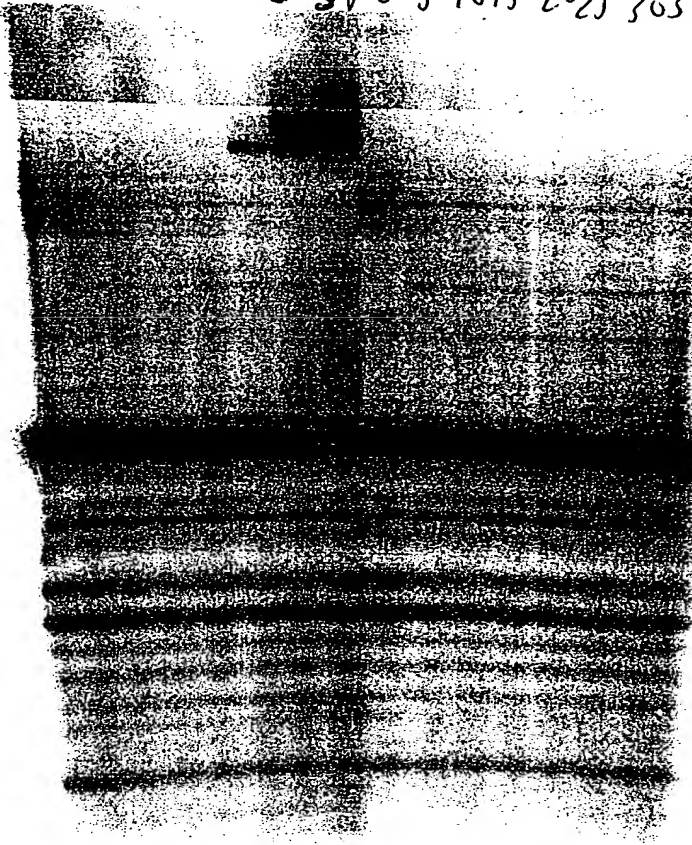
Tag N _____

PAGE

Tf1 units 1.33

4u

0 5 10 15 20 25 30 36 0 5 10 15 20 25 30 36 cycles

O/N
experiment100 -
100, 100
gray

Result
only slight degradation (< 1 nt/primer) after
36 cycles
note formation of primer dimer cycles 25-36
but only for low Tf1!

To Page No. _____

Read & Understood by m ,

Date

11/29/94

Invented by

Rec rd by

Date

10-27-94

Project No. _____

Book No. _____

TITLE Q₆SDM . Tosohakos .

14

rom Pag No. _____

Used DEPC treated water to make all buffers from now on out -

Washed column + column matrix extensively with
.5M NaOH -

Poured a 40 mL Q₆SDM - 8cm x 2.5cm - col
Wash w/ .5M NaOH
Wash w/ 1L of DEPC treated sterile H₂O
Wash & Equilibrate w/ Buffer A

Buffer A -

25mM KPO₄ pH 7.2
10% glycerol
10mM KCl
5mM Bme
1mM PMSF

Buffer B -

25mM KPO₄ pH 7.2
10% glycerol
800mM KCl
5mM Bme
1mM PMSF

↓
initial conductivity 3² mS

50mS

Sample conductivity 4mS - dialyzed in buffer A - ~ 27.5 mL - cor
from dialysis

Program -

Load 0.5 mL/min .

Wash w/ 120 mL of Buffer A 1 mL/min - collect 7.5 mL fractions 2.

Gradient - 400 mL linear gradient Buffer A - Buffer B - 1 mL/min " 10

Wash w/ 120 mL of Buffer B 1 mL/min collect 7.5 mL fractions -

To Page N

Witnessed & Understood by me,

Mary Longo

Date

4/5/95

Invented by

E. Hyman

Recorded by

Date

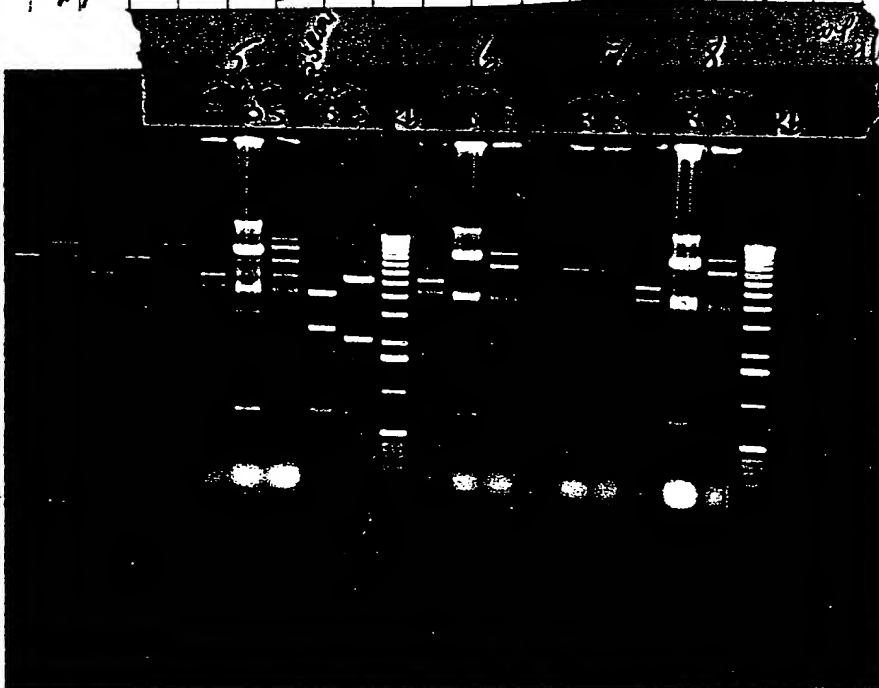
4/2/95

ig N

2/18/95 wed

Con'd from 3884 NB
MINI PREP DNA

- cfg 500 μ l of cells for 1 minute in an eppendorf cfg (centrifuge)
- removed supernatant and resuspended pellet in 100 μ l of 1X PEBI (SI) ^(saved)
- added 200 μ l of alkaline - SDS mix
- placed the tubes on ice for few minutes (3-5 min)
- added 150 μ l of 7.5 M Ammonium Acetate
- Mixed the tubes by inverting
- cfg the tubes for \sim 7-10 min.
- transferred 400 μ l supernatant to the new eppendorf tube
- added 800 μ l of ethanol to supernatant. Mixed tubes.
- incubated the tubes for \sim 2 min. Spin.
- dissolved pellet in 50 μ l of TE + RNase A.
- applied 5 μ l to a 1% agarose gel.



SI = 0.9% glucose
25 mM Tris HCl (pH 8.00)
10 mM EDTA

alkaline - SDS mix = 1% SDS
0.1 N NaOH

To Page No. _____

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Date

Invented by

Dat

Recorded by

4/12/95

From Page No.____

* AAT 11 * 1 : G C C A C C T G A C G T C T A A G A A A C C A
 non du 3, mea. TAT TAT C

* AAT 1 # 2 : GTT TCT TAG ACG TCA GGT GGC ACT TTT
now du 29 meen

* AAT $\overline{11}$ * 1 : Gec Ace UGA CGA CUA AGA AAC CAU TAT
du 31 mer...

* AAT II #2 : GTT TCT UAG ACG UCA GGU GGC ACC
du 29 meu TTT

Purpose To try to amplify pMC9 with these new primers

increased the annealing to 58° from 56° (previous exp)

enzyme : Tag alone (10)
Tag + Descent (1 + 1.01)
D.V. (0.5 + 1)

premiers : 1 non du } ne
2 du }

→ done in duplicate.

4 1 dev 2 no dev

12. Rx / enzyme - can pre mix med.

$$5. \quad 2 \, du + 1 \, w \, dw$$

- Deep vent buffer was used there and

| | | |
|-------------|------------------|-------|
| <u>Tag:</u> | supper | 60 |
| | dinner | 12 |
| | Terry's milk | 2.4 |
| | enzyme | 2.4 |
| | H ₂ O | 403.2 |
| | | <hr/> |
| | | 480 |

Tube # (L-11)

200 μM d.n.t.
200 μg Temp
1 μM pressure
2 mM Mg
comes w. buy

40 µl / Rx = aduad def. primer en 10 µl .

To Page No.

With ssed & Understood by m ,

Date 11/28/24

Invented by

Date _____

Recorded by

7/23/70

ag N _____

primers old } = 20x : ~~2.5~~ 10 + 10 ml of each primer at (100 µl)
 do }
 180 µl 11/20

primers do } = } equivalent amount of each
 new }
 other 2 different combos } 50 + 50 ml (10 µl each)
 1 do + other new do }

2 V: buffer 60
 dNTP 12
 01) Temp 2.4
 me elongase 12.0 (1.01/µl)
 H₂O 398.6
 480.0

2 V alone 0.5 µl 1.0
 buffer 60 60
 dNTP 12 12
 Temp 2.4 2.4
 20/µl elongase 3.0 6.0
 H₂O 402.6 399.6

ube % (12 - 22)

(23 - 33)

(33 - 44)

11, 22, 33 & 44 w/o any primers.

cycling: 94°, 3'

30(94°, 30", 50°, 30", 72°, 2') → 4° soak

samples thrown out 12/19/94

To Page No. _____

Used & Understood by me,



Date



Invent d by

Recorded by

K. Sitarman

Dat

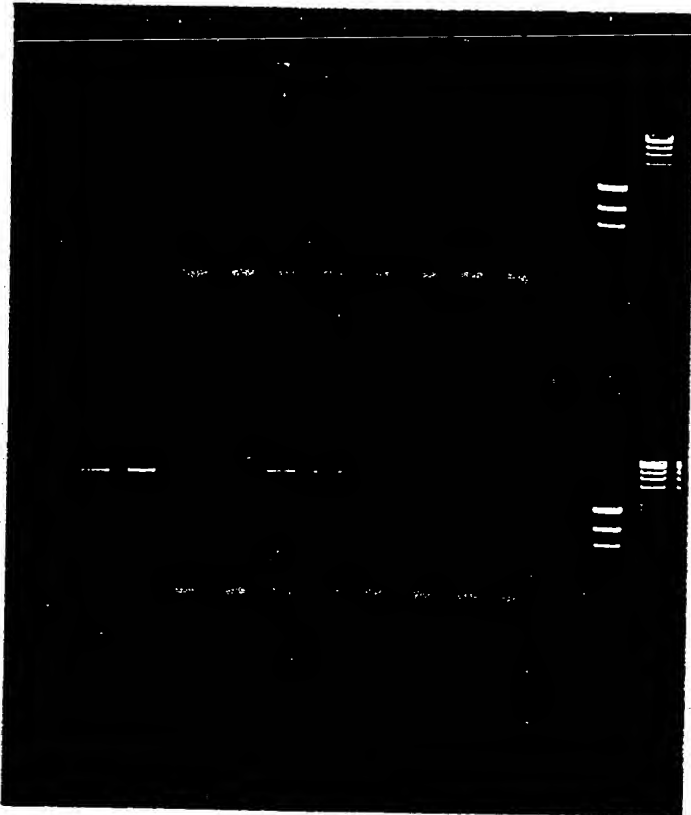
11/23/94

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Book No. _____

TITLE _____

m Page N _____

Tag:

$N + N + 0 + -/+ -/+$ no primer
 Tag + D.V. 1 : 0.01 :

Deep vent as usual
didn't work →

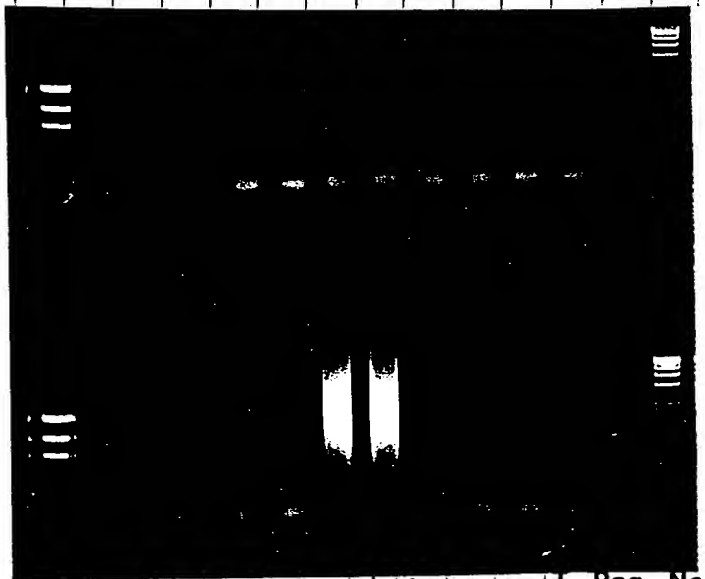
with old d.v. primer smears
for the first time.

Why more primer dimer
with d.v. - than with
non d.v.?

Result:

- Even with Tag problem with product at the annealing temp.
- F/R - no d.v. product as slightly with Tag, more with T + D.V.
- old primer at 50° did work with Tag.
- with Tag + D.V. misprimed all most gone with old primer new misprimed product with T + D.V.
- mismatch didn't work at all.

D.V. 0.50



ness d & Understood by me,

Date

Inv nted by

Date

Record d by

Pag No

11/23/94

11/23/94

sk A.K. ...

Project No. _____
B ok No. _____

183

TNE

ag N

lig

TQ19 / SmaI / SphI .003 pmol/.1
x6 H3 / Filled in / SphI .015 pmol/.1
5X ligation buffer
H₂O
Ligase (10)

2
1.5
1
4
12.5
1
20.1

RT - 30 min.

Jason xformed 2 ul of the lig with 100 ul DH10B cc.
std xform. Plated 10% + 90% on yet amp plates. 37°C ON

#2 10% 90%
18 ~150

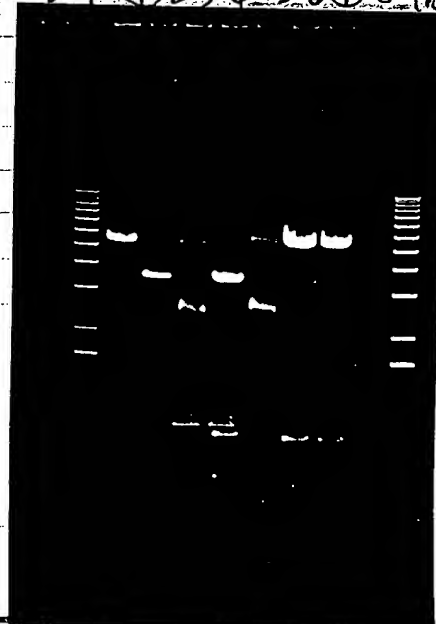
picked 8 colonies into 3 mls of CG + amp 100. 37°C - ON

mp as usual. Dissolved in 50 ul TE

mp 3
IDRL 2
H₂O 13
BpH 1
E. coli 1
10

sub PUL TNE 35 PY mut into
SmaI / SphI site of pTQ19
clones cut E SmaI / EcoRI
2 kb 1 2 3 4 5 6 7 8 1 kb

ANY 8/1/95



37°C - 1 hr.
Applied to a
0.9% agarose
gel. Gel
run at 100V

4875
2000
6575

To Page No. _____

sed & Understood by me,

Date

Inv nted by

Date

8/3/95

Recorded by

8/13/95

Lisha Xin

Colony Long

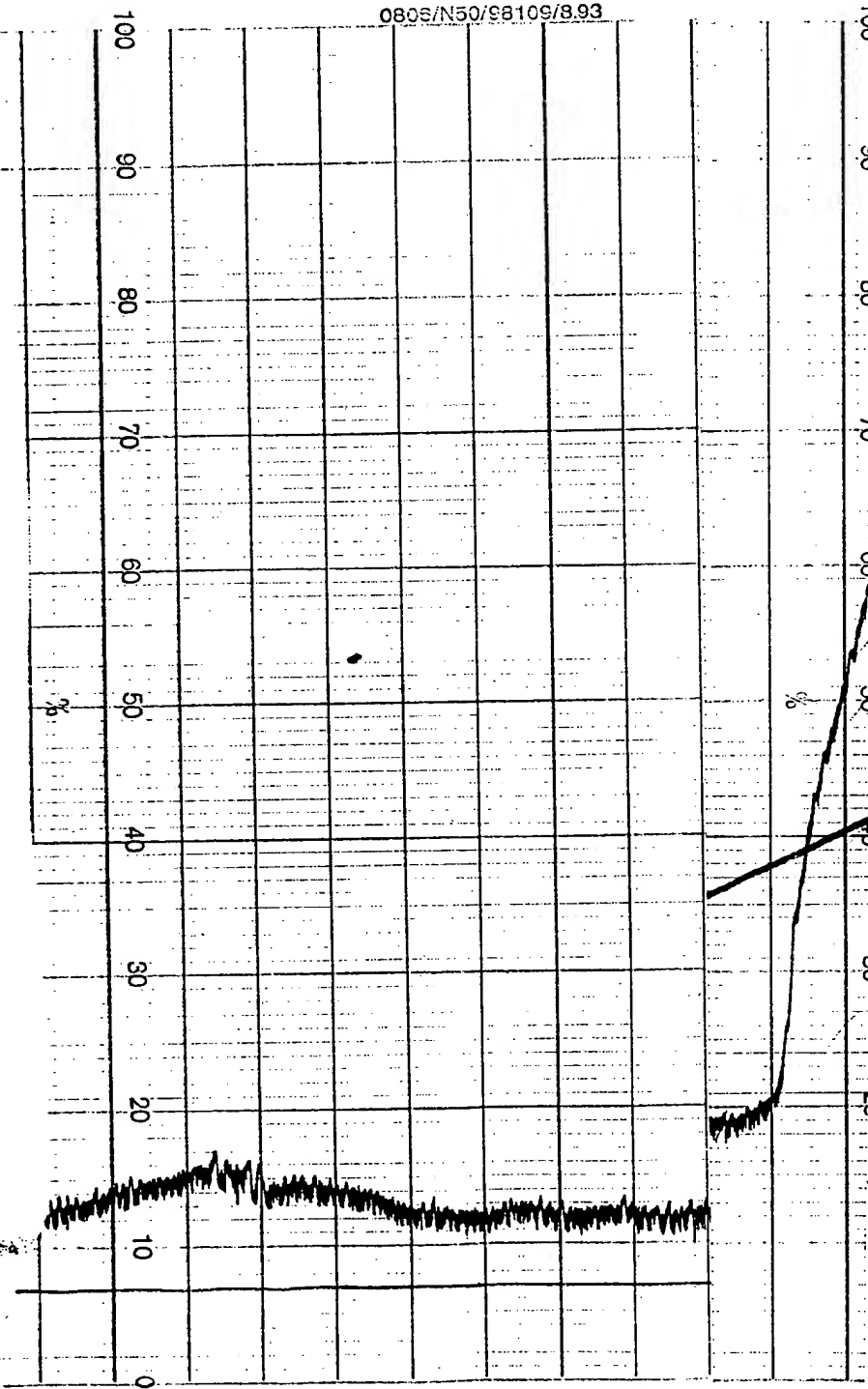
Project No. _____
Book No. _____

115

Deparment Q 650 Tne-Durification

Page No. _____

0805/N50/98109/8.93



24 04/03/95

gmm 4/5/95

To Page No. _____

Read & Understood by me,

Date

Invented by

Date

May Longo

4/5/95

Recorded by

04-103/95

Project No. _____
Book No. _____ TITLE _____

2

From Page No. _____

2/9/95 th

Purification of m13 ssDNA

1. cfg 1.0 ml of *infected cell culture for 2 min. (1 to 5 m)
2. Transferred 800.0 μ l to the new tubes
(Pellet was saved for isolation of RF DNA)
3. cfg supernatant again to remove any residual cells
4. added 200.0 μ l of 20% PEG + 1.5 M NaCl. Vortexed
5. Incubated tubes at room temperature for 5 min.
6. cfg tubes for 5 min. & discarded supernatant (sup.)
7. added 200 μ l of *TE & vortexed really good.
8. cfg for ~ 1-2 min. (to remove any residual cell debris)
9. transferred sup. to the new tubes. (RNaseA can be added here)
10. added equal vol. of phenol/chloroform/isoamyl alcohol
(25:24:1) Mixed well.
11. cfg 5 min.
12. removed the aq (upper) layer to a new tube (be very careful)
13. added $\frac{1}{10}$ vol. of 3M NaAc + $2\frac{1}{2}$ -3 vol. of 95%.
14. Incubated @ -70°C till 2/14/95.

{ 20.0 μ l Na6
600.0 μ l ET

TE (T₁₀E₁) = 10 mM Tris-HCl pH8.0 + 1 mM EDTA pH8.0

infected cell culture = ① grew an E. coli F' strain to an OD of 0.4 in 2xYT
↓
F' = Fertility Factor: codes for tra genes & pilis to allow infection of the
m13 Phage. (transfer of DNA)

Cont'd - - - To Page No

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Date

Invented by

Date

Recorded by

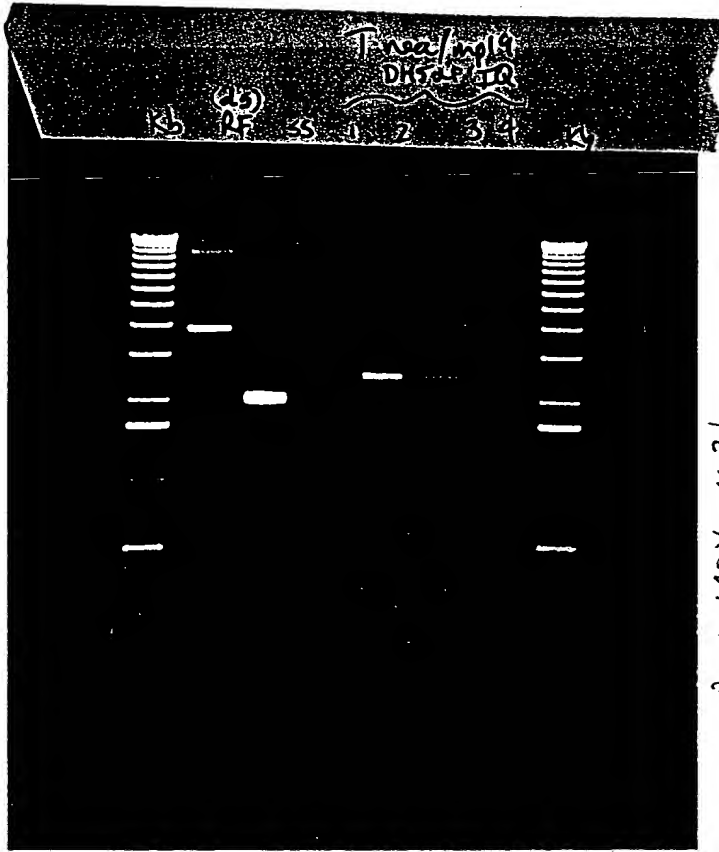
4/12/95

Project No. _____

Book No. _____

5

Page No. _____



RUN 140V ~ 2 hrs.

amp 4/12/95

To Page No. _____

Read & Understood by me,

J. Polansky

Date

4/12/95

Invented by

Recorded by

[Signature]

Date

4/12/95

From Page No. _____

8/1/95

lig

PTTQ19/SmaI/SphI .003 pmol/ μ l
 2 kb HB/Filled in/SphI .015 pmol/ μ l
 5X ligation buffer
 H₂O
 Ligase (10)

| |
|------|
| 2 |
| 1.5 |
| 1 |
| 4 |
| 12.5 |
| 1 |
| 20.1 |

RT - 30 min.

Jason reform 2 μ l of the lig with 100 μ l D4103 cc.
 std reform. Plated 10% + 90% on yet amp plates.

8/2/95

#2

10%
1890%
~150

picked 8 colonies into 3 ml of CG + ampic. 37°C -

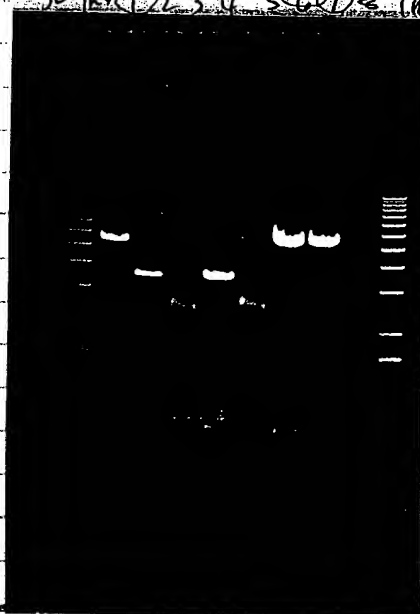
8/3/95

mp as usual. Dissolved in 50 μ l TE.

mp 3
 D4103 2
 H₂O 13
 BpH 1
 EcoRI 1
 10

sub PUCTNE 35 PY mot into
 SmaI/SphI site of PTTQ19
 cloned site SphI/EcoRI
 5 kb 2.3 kb 3.6 kb 3.1 kb

ANY



Vector 4575
 insert 2000
 6575

37°C - 1 hr.
 Applied to a
 0.8% agarose
 gel. Gel
 run at 180V

To Page No. _____

Witness d & Und rstood by m ,

Lisa Xu

Dat

8/3/95

Invented by

Recorded by

C. M. L. L. L.

Date

8/13/95

annealing temperature

Project N

pmc9 / dif. enzy / dif. primer / dif

Book No.

11/28/94

109

Tag No.

purpose: To check at different annealing temp to get rid of mispriming during pmc9 amplification

Temp checked 58°, 60°, 62°, 65°

56° gave more non specific bands than 58°. pg 102 -

checked all three primer set { Add - II new dv
 " " " - dv
 " old dv 2728 + 29

Amplified with Tag, Tag + DV, DV alone.

200 µM dNTP
 1 µM primer
 200 µg template
 2 mM buffer (from buffer) - used Deep Vent buffer

prepared cocktail for 80 Rx

added primers separately, adding respective enzymes.

| | | | | |
|--------|---------|-----------|--------|-------------------------------|
| well # | 1 - 8 | Tag | old dv | each combination |
| | 9 - 16 | | New dv | in duplicate |
| | 17 - 24 | | " - dv | annealed at different |
| | | | | temp 58, 60, 62, 65° |
| | 25 - 32 | Tag + DV | old dv | |
| | 33 - 40 | | new dv | 94° 3' |
| | 41 - 48 | | " - dv | |
| | | | | 30 (94°, 30", X 30", 72°, 3') |
| | 49 - 56 | Deep Vent | old dv | |
| | 57 - 64 | | new dv | 72° 10' |
| | 65 - 72 | | " - dv | |
| | | 15 U | | 4° 20 min. |

To Page No.

ss d & Understood by me,

[Signature]

Date
 12/19/94

Invented by

Recorded by

[Signature]

Date

11/28/94

Project No. _____

Book No. _____

TITLE _____

110

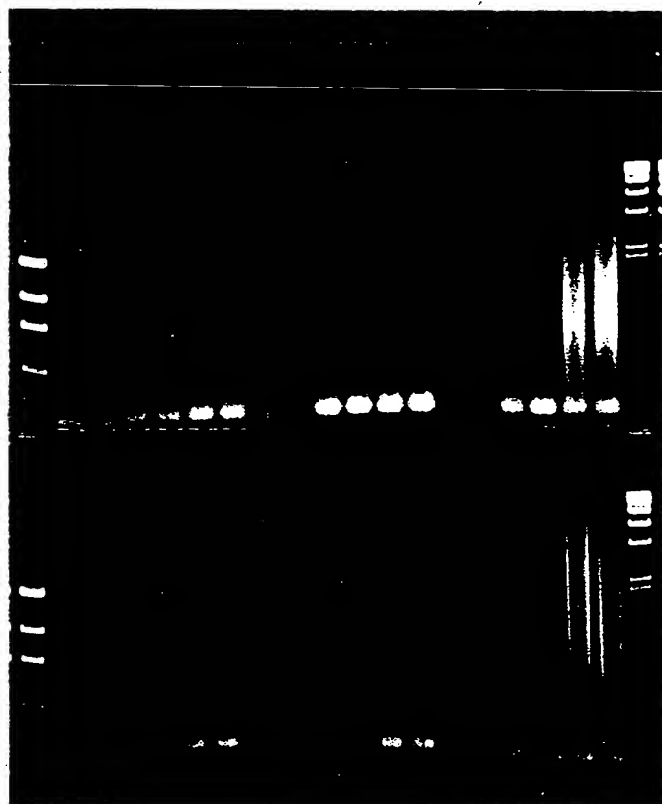
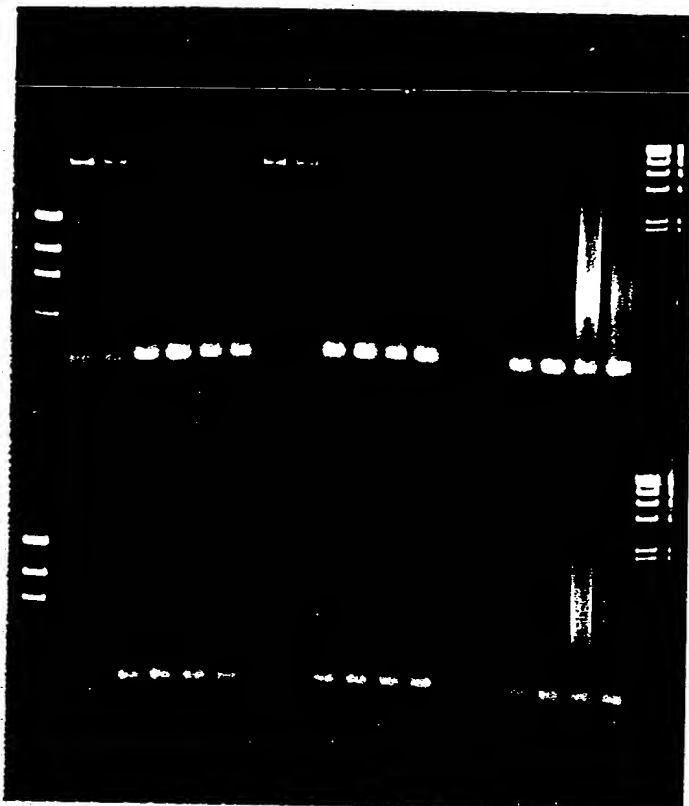
From Page No. _____

Tag
0-30 N. 40 W.

Tag, D.V.

D.V.

58°

Result:

The only thing that worked,

Tag

Tag + D.V.

old dv primers at 58°

Deep Vent / non dv / at all anoxic temp remains?

All samples discarded

To Page N

Witnessed & Understood by m,

Date

12/18/94

Invented by

Recorded by

sk. Mike ...

Date

11/29/94

70

Project No. _____

Book No. _____

TITLE _____

From Page N _____

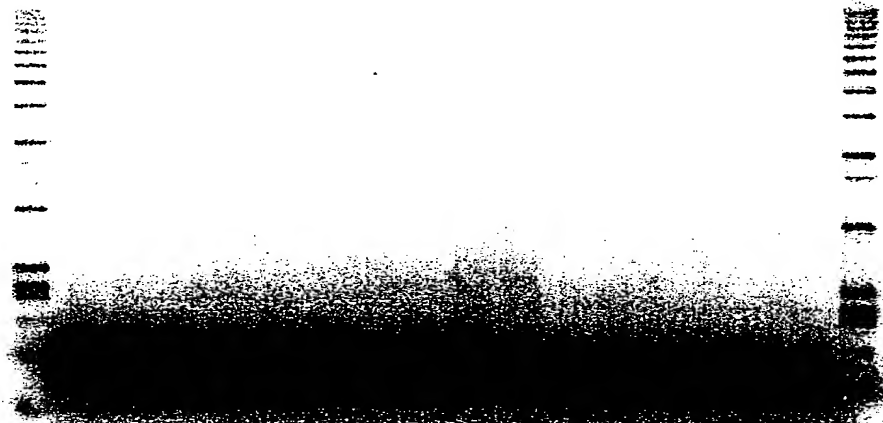
Tf (min)

1.33

4

Age

10 5 10 15 20 25 30 36 cycles



O/N spread 100 - 100,000 gray

Result: no 13.5 kb product - maybe too much Mg
with hot primer!
nonspecific smear ~~also~~ seen with E+Br (B69) is
cold in auto rad ~~above~~ above

Witnessed & Understood by me,

Date

Invented by

Date

To Page

11/29/94

Recorded by

10-27-94

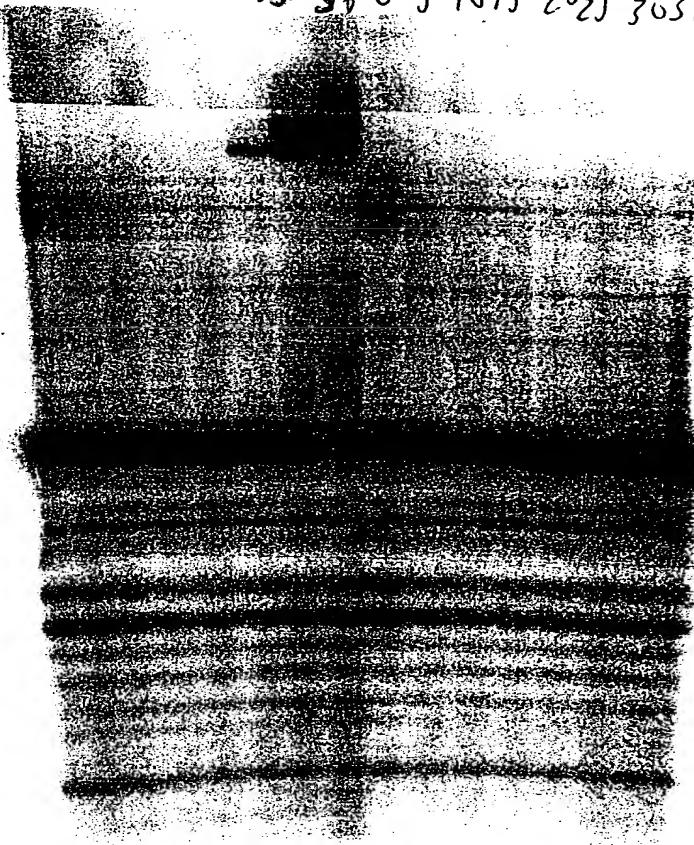
Deena Golay

PAGE

Tf1 rate 1.33

4u

0 5 10 15 20 25 30 36 0 5 10 15 20 25 30 36 cycles

O/N
experiment

100 -

100, 100

gray

Result:
only slight degradation (< 1 nt/primer) after
36 cycles
note formation of primer dimer cycles 25-36
but only for low Tf1!

T Page No. _____

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Date

Inv nt d by

Date

11/29/94

Record d by

10-27-94

74

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

make 2.5 μ l rTag (EXBT1 lot) by 1:1 dilution
of 5 μ l (on P 61) with storage buffer:

5 μ l rTag P 61

20 μ l

storage buffer

20 μ l

VP 40 μ l

To Page N

Witnessed & Understood by me,

Deborah Pokup

Date

11/29/94

Inv. nted by

Rec. rded by

Date

10-27-94

Project No. _____

Book No. _____

TITLE Units - on Loads + Pools -

118

From Page No. _____

Purpose: What to determine total units on Heparin + Q650 + the total units pooled + determine units / gram from crack sample

1. crude - $\frac{1}{2000}$
2. after heat shock $\frac{1}{2000}$
3. Load PET.
4. Load Hep $\frac{1}{1000}$
5. Pool Hep $\frac{1}{1000}$
6. Load Q650 $\frac{1}{1000}$
7. Pool (1) Q650 $\frac{1}{500}$
8. Pool (2) Q650 $\frac{1}{500}$

(7x3) = 21 samples -

Tag Dislution Buffer
25mM Tris pH 8.0
80mM KCl
100 μ g/mL glycine
1mM EDTA
.5% NP-40
.5% Tween 20
1mM Bme

| SAM | CPM1 | |
|---------------------|----------|--------------|
| 1 $\frac{1}{500}$ | 1958.00 | Load Hep 118 |
| 2 $\frac{1}{500}$ | 2486.00 | 42 |
| 3 | 3196.00 | 48 |
| 4 $\frac{1}{500}$ | 2746.00 | 49 |
| 5 $\frac{1}{500}$ | 3998.00 | 7234 |
| 6 | 5108.00 | 23 |
| 7 $\frac{1}{500}$ | 3000.00 | 72 |
| 8 $\frac{1}{500}$ | 4990.00 | 60 |
| 9 | 5510.00 | 33 |
| 10 $\frac{1}{1000}$ | 4888.00 | 59 |
| 11 $\frac{1}{1000}$ | 7964.00 | 48 |
| 12 | 8240.00 | |
| 13 | 7990.00 | |
| 14 | 10032.00 | |
| 15 | 8612.00 | |
| 16 | 428.00 | |
| 17 | 78186.00 | |
| 18 | 78040.00 | |
| 19 | 79558.00 | |
| 20 | 22.00 | |
| 21 | 26.00 | |

78594.7
SA = 49.7

Not too good need to rean

4/5/95

To Page N

Witness d & Understood by m ,

Date

Invent d by

Date

May Longo

4/5/95

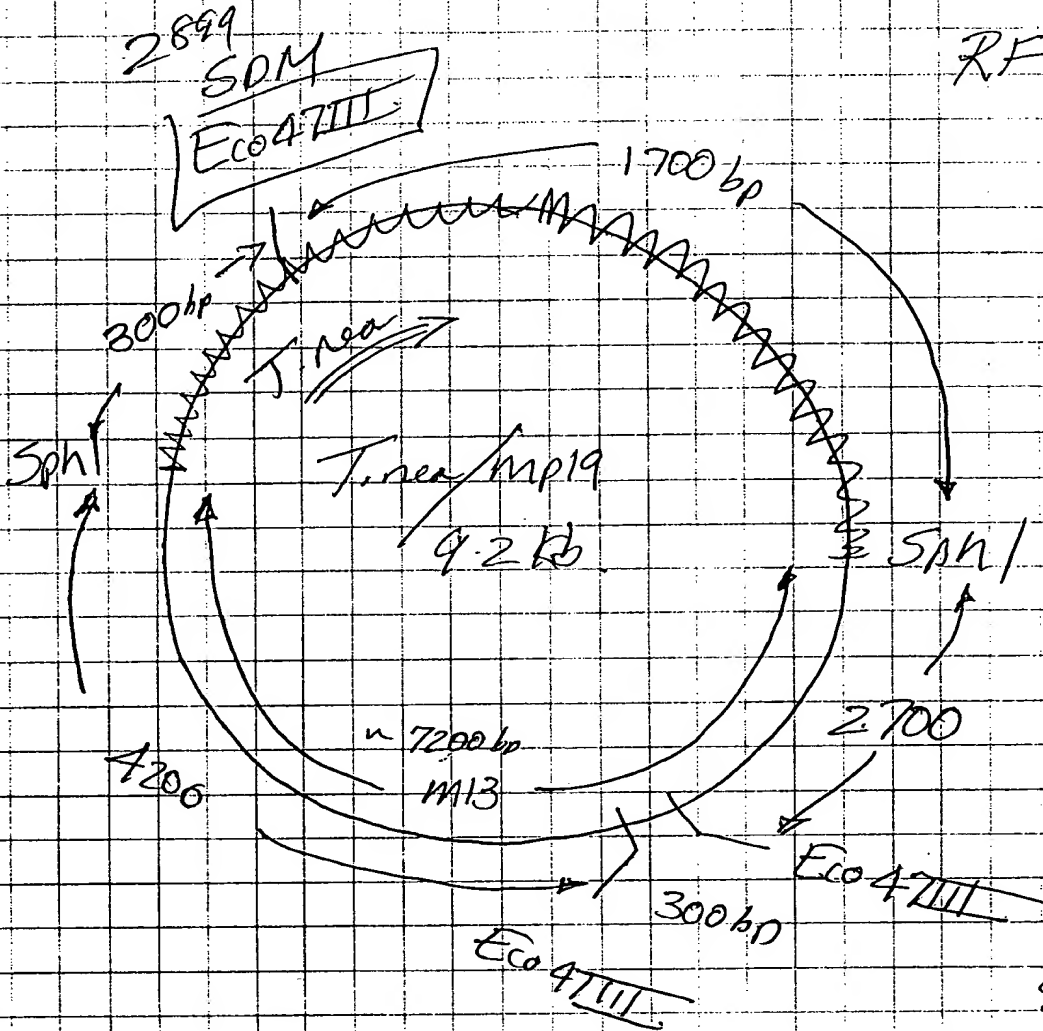
Rec rd d by

04/04/95

Page No. _____

SDM 2899

RF map



See on file

| | Eco 47III | 1 kb | PARENT | MUTANT |
|--------|----------------------------|--|--------|--------|
| PARENT | 8.9 Kb
0.3 Kb | 5 kb
4 kb
3 kb
2 kb
1.6 kb
1 kb
500 bp | ===== | ===== |
| MUTANT | 4.5 Kb
4.4 Kb
0.3 Kb | | | |

may be too light to see

| | | | |
|---|---------------------|------------------------------------|---------------------|
| Read & Understood by me,

May Jorja | Date

2/16/95 | Invented by

Dr. J. J. Jorja | Date

2-16-95 |
| | | Recorded by

Dr. J. J. Jorja | |

Page No. _____

The samples from previous expt were run on new Tablets
received from Jim Spencer. (11/28/94) agarose.

1 Tab = 1 gm

Dissolved 2 gm in 200 ml of 1X TAE (0.2 mM EDTA)

Began to dissolve in few minutes at RT in buffer,
looked like powder agarose (regular) in buffer.

microwaved for 4-5' (3' didn't completely go into soln)

added 5 µl of 10 mg/ml ethidium bromide

easy to pour, no bubbles } like ThermoGel - looked
not dense } more like regular agarose

when solidified looked a bit transparent than regular
agarose, well formed wells.

The gels were even at 100-105 V constant, along with
11x14

DNA mass ladder and Hind III / Lambda.

Looked like it ran a bit faster than regular agarose.

Ladders resolved quite well, the intensity of bands
in mass ladder looked normal.

Apart from convenience of no need to ~~weigh out~~,
there is no other added advantage.

maybe this gel is slightly faster than regular agarose
Is it really 1%?

To Page No. _____

Used & Understood by me,



Date

12/9/94

Invented by

Recorded by

K. Sitararaman

Date

11/29/94

54P primer for Vent digestion
and 25 ad/or ribo ends

Same as
P128, 6

| ag N | | | | | | | |
|----------|---------------|----|-----|-----|-----|-----------------|-----------------|
| mer | 0.66 μ M | ✓✓ | 8.1 | | ✓ | 23 min. work is | 0.66 pm / 1 min |
| (5 ng/l) | | | | | | | |
| 7.1 | 14.6 μ M | | | | | | |
| 6.6 in | = 1 μ M | | 6.1 | | ✓ | | - - |
| 7.2 | 10.9 μ M | | | | | | |
| 5.9 | = 1 μ M | | 6.1 | | ✓ | | - r |
| 7.6 | 38.6 μ M | | | | | | |
| 5.6 | = 1 μ M | | 6.1 | | ✓ | | S - |
| 7.7 | 59.36 μ M | | | | | | |
| 59.36 | = 1 μ M | | | 6.1 | ✓ | | S r |
| 1.0 | 11.6 μ M | | | | | | |
| 1.8 | = 1 μ M | | | | 6.1 | ✓ | - S |

| | | | | | |
|------------------|-----|-----|------|---|---|
| Rinse buff | ✓✓✓ | 4 | 2 | → | ✓ |
| 3.4P ATP 3.450 | ✓✓ | 4 | 2 | → | ✓ |
| 11-4-94 ref | | | | | |
| PKK | ✓✓ | 1 | 9.5 | → | |
| H ₂ O | ✓✓ | 2.9 | 10.6 | → | ✓ |
| rf = 20 μ l | | | | | |

37°C 30 min, 55°C, 5'

| | | | | | |
|-------------------------|--|----------|-------------|---------|------------|
| s d & Understood by me, | | Date | Inv nted by | Dat | To Pag No. |
| maea Polamp | | 11/29/94 | | 11-1-94 | |
| | | | Rec rd d by | | |

Project No. _____

B ok No. _____

119

SAM CPM1

| | | | |
|----|----------|------|---------------------------|
| 1 | 4148.00 | 93 | Load
Heparin |
| 2 | 4852.00 | 55 | |
| 3 | 6730.00 | 40 | |
| 4 | 2580.00 | 42 | Post Heparin
Load Q450 |
| 5 | 3952.00 | 34 | |
| 6 | 5700.00 | 25 | |
| 7 | 5318.00 | 31.7 | Pool
Q450 |
| 8 | 3176.00 | 38 | |
| 9 | 2294.00 | 55 | |
| 10 | 3002.00 | 34 | Pool |
| 11 | 8568.00 | 33 | |
| 12 | 5524.00 | | |
| 13 | 1742.00 | | |
| 14 | 1812.00 | | |
| 15 | 4872.00 | | |
| 16 | 6352.00 | | |
| 17 | 242.00 | | |
| 18 | 82428.00 | | |
| 19 | 81076.00 | | |
| 20 | 77332.00 | | |
| 21 | | | |

5.80 u/ml 4.75 positive control
3.7

$\bar{x} = 80278$

SA = 580 cpm/nmol

Pooled together the two
pools from Q450 - 16.5 mL
added .5% w/v
of TritonX + NP-40

Adams premix + 1.1 mL ~~ADAMS~~ ^{dd CTP}
48 uL of g premix
added to pre-labeled
effluents - 1, 2, 4, 8
of diluted sample
was added - incubated
for 10 minutes at
74°C - the rxn was
quenched w/ 10 uL
g. 5 M EDTA +
10% -
30 uL was spotted on
6 FIC filters -
TCA wash + EtOH wash
dried & counted.

alldr tubs made in serial

$$\left(\frac{10}{100} \right) \left(\frac{1}{x} \right)$$

$x = 200, 150, 100,$

| | U/uL | Total Units | Vol. | mg/mL | total mg | SA | 1/gm cell |
|-------|---------|---------------------|-------|-------|----------|--------------------|-------------|
| AS1 | 62 u/uL | 1.3×10^4 | | 1.4 | 30 | | |
| Load | | 1.3×10^4 | 21 mL | 1.4 | 30 | 4.3×10^4 | |
| Pool | 39 u/uL | 1.07×10^4 | 27.5 | | | | 77% recover |
| ad | 39 u/uL | 9.675×10^5 | 25 | .323 | 8.0 | 1.22×10^5 | 3% pur. |
| ol/ | | | | | | | |
| algas | 38 u/uL | 6.27×10^5 | 16.5 | | | | 65% recover |

very conservative ~ 20,250 u/gram cell - for 10 cells - 500 gram crack

To Page No. _____

ssed & Underst od by me,

May Longo

Date

4/5/95

Invented by

Recorded by

Date

04/05

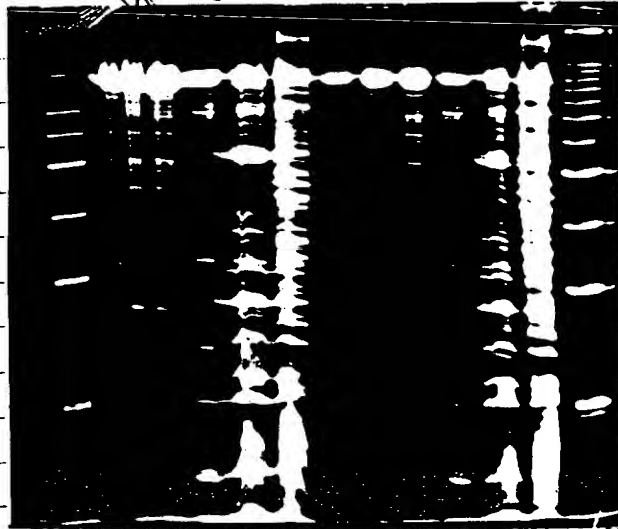
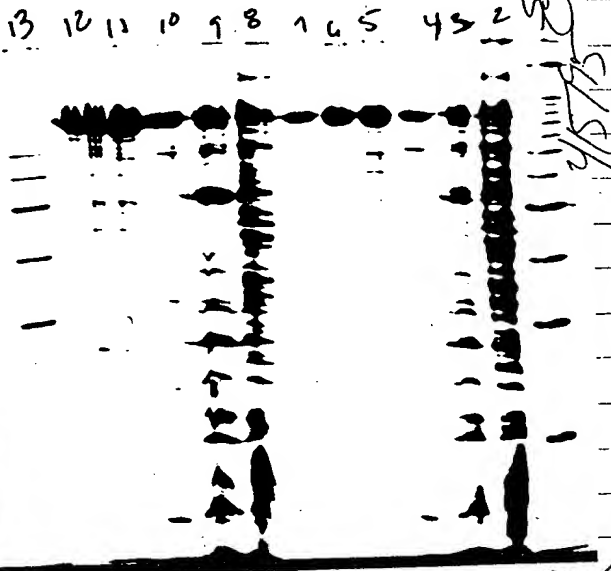
Project No. _____

Book No. _____

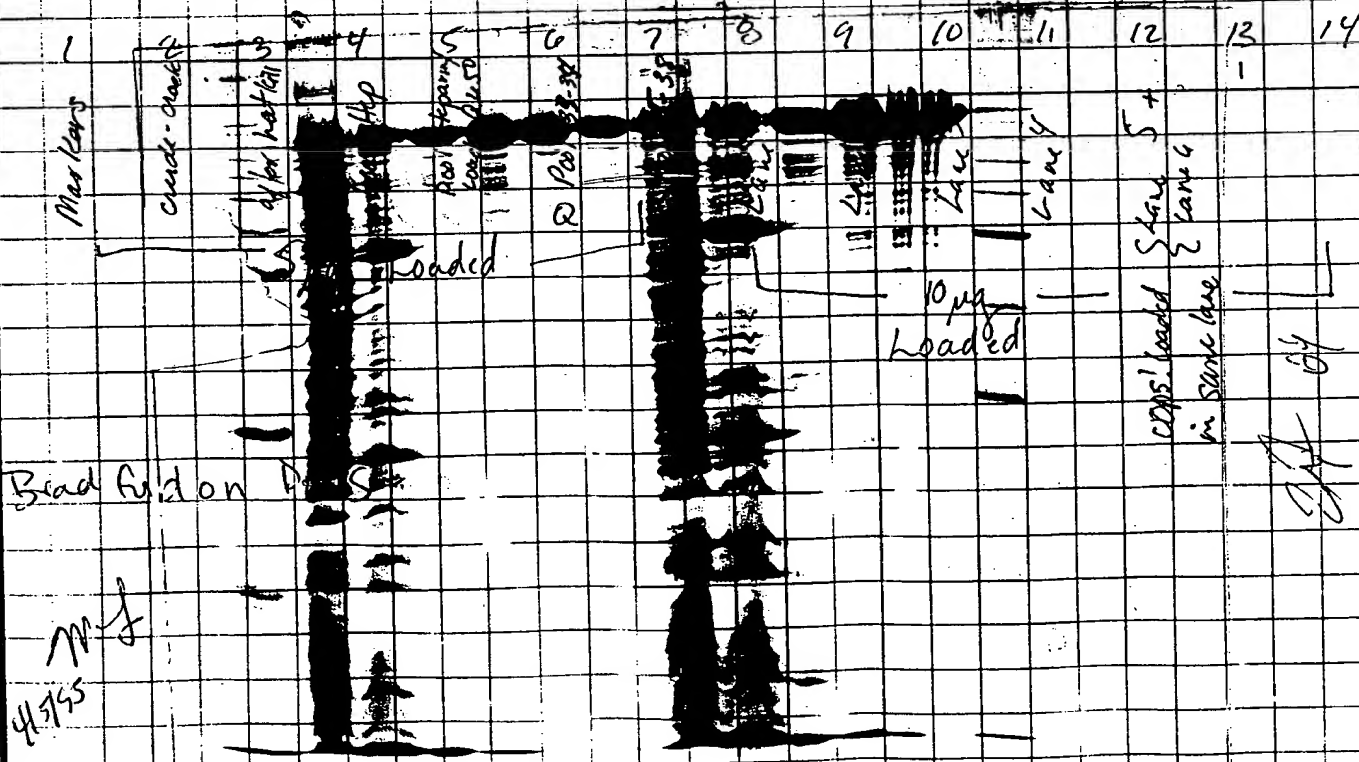
TITLE

Gal of Pods - 12.5% PHGE

From Page No. _____



LIFE TECHNOLOGIES, INC.



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May Tongo

Date

4/5/95

Invented by

E. Myer

Recorded by

Date

04/05/95

To Page

QC. RNase Assay -

Project No. _____

Block No. _____

12

Page No. _____

| Tube | Rxn mix | Enzyme Unit | μ l H ₂ O |
|------|------------|-------------|--------------------------|
| 1 | 50 μ l | 2 | 4 μ l 8.5 μ l |
| 2 | | 5 | 1 μ l 3 4 |
| 3 | | 10 | 2 μ l 2 3 |
| 4 | | 15 | 3 μ l 2 |
| 5 | | 20 | 4 μ l 1 |
| 6 | | 0 | 5 μ l Dil'n Buffer |
| 7 | | 0 | 5 μ l DEPC |

Dilute Enzyme - 1/5

$$\frac{190 \mu\text{l Tris}}{950 \text{ total volume}} = \frac{190}{(950-190)} = \frac{190}{760} \mu\text{l dil'n Buffer}$$

$$\text{dilute to } 50 \mu\text{L} = \frac{1}{7.6} = \frac{10}{76} = \frac{10}{(76-10)} = \frac{10}{66} \text{ enzyme}$$

Rxn mix

Tag premix

PCR mix

10x Buffer

A.G.

8 μ l

mRNA GluRin
processed H₂O

160 μ l 95% 320 μ l 8 μ l
232 μ l 72 μ l 8 μ l

400 μ l

Incubate at 37°C in heat block
for 1 hour -

Add 4 μ g Proteinase K (2mg/mL) + 2 μ l
25 μ g tRNA (5mg/mL) + 2.5 μ l

Incubate 10 min @ 37°C

Add 20 μ l 2M NaAc + 200 μ l 100% EtOH - vortex
Keep in freezer - 20°C O/N

To Page No. _____

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Man Longo

4/5/95

Recorded by

4/5/95

Project No. _____

Book No. _____

TITLE _____

6

From Page No. _____

2/15/95 Wed.

(+) strand (ssDNA) lot # ED5702 260 μ g/ml
RF strand (dsDNA) lot # CC 3.111 5 μ g/18.4 μ l

calculation: ssDNA = 260 μ g/ml = ng/ μ l

$$\frac{260 \text{ } \mu\text{g/ml}}{1000 \text{ ng/} \mu\text{g}} \cdot 1000 \text{ ng/} \mu\text{g} \cdot \text{ml} \cdot 1000 \text{ } \mu\text{l} = 0.260 \text{ } \mu\text{g/} \mu\text{l}$$

$$\frac{1000 \text{ ng/} \mu\text{g} \cdot (0.260 \text{ } \mu\text{g})}{260 \text{ ng/} \mu\text{l}} = 260 \text{ ng/} \mu\text{l}$$

$$\frac{260 \text{ ng/} \mu\text{l}}{2.6} = 100 \text{ ng}$$

$$\left\{ \begin{array}{l} 260 \left(\frac{1}{2.6} \right) = 100 \text{ ng} \\ \text{or} \\ \frac{260}{2.6} = 100 \text{ ng} \end{array} \right.$$

for 2.6 total or final volume
you need 1.0 μ l DNA

$$\begin{array}{r} 1 \text{ } \mu\text{l DNA (260 ng/} \mu\text{l)} \\ 1.6 \text{ } \mu\text{l TE} \\ \hline 2.6 \text{ } \mu\text{l} \end{array}$$

for 100 ng/ μ l, } 2.0 μ l DNA (260 ng/ μ l)
multiply by 2 } 3.2 μ l TE

dsDNA = 5 μ g/18.4 μ l.

$$1000 \text{ ng/} \mu\text{g} \times 5 \text{ } \mu\text{g} = \frac{1000 \text{ ng} (5 \text{ } \mu\text{g})}{\text{ } \mu\text{g}} = 5000 \text{ ng/18.4 } \mu\text{l}$$

$$\frac{5000 \text{ ng}}{18.4 \text{ } \mu\text{l}} = \frac{272 \text{ ng/} \mu\text{l}}{2 \text{ } \mu\text{l}} = 2.72 \text{ ng}$$

for 2.7 total volume you need
1.0 μ l DNA

Total Volume (TV)

To Page 1

With ss d & Understood by me,

Date

Invent d by

Dat



4/12/95

R c rded by

4/12/95

Tag No. _____

| | Tube # 1 | | | Tube # 2 | | |
|-----|-------------|------------|---------------|----------|-------------|------------------------|
| | RF (ds) | | | ⊕ ssDNA | | |
| DNA | 1.0 μ l | $\times 3$ | $= 3.0 \mu$ l | DNA | 1.0 μ l | $\times 3 = 3.0 \mu$ l |
| TE | 1.7 μ l | $\times 3$ | $= 5.1 \mu$ l | TE | 1.6 μ l | $\times 3 = 4.8 \mu$ l |
| TV | 2.7 μ l | $\times 3$ | $= 8.1 \mu$ l | TV | 2.6 μ l | $\times 3 = 7.8 \mu$ l |

Tube # 1, 2, 3, 4 of RF (dsDNA)

| | ① | ② | ③ | ④ | |
|------------------|--------------------------------------|----------|-----------|--------|---------------------------------|
| | Alu I | Hind III | Sau 3 A I | Bam HI | |
| H ₂ O | 16.0 μ l | | | | → (all 4 tubes w/ 16.0 μ l) |
| 10x Buffer | 2.0 μ l | | | | |
| DNA | 1.0 μ l | | | | |
| | (React 1; React 2; React 4; React 3) | | | | |
| Alu I | + | - | - | - | |
| Hind III | - | + | - | - | |
| Sau 3 A I | - | - | + | - | |
| Bam HI | - | - | - | + | |

Tube # 1, 2, 3, 4 of ⊕ (ssDNA) same order as RF

2 tubes were set-up for uncut, 1 with RF & 2nd with ⊕

- each tube added 16.0 μ l H₂O
- 2.0 μ l REact 2 10x buffer
- 1.0 μ l DNA

• Put all 10 tubes in

• ran the sample (all 10) on a gel next morning.

(0.8% agarose gel, 147 volts)

• picture of the gel is on the next pg (pg # 8)

To Page No. _____

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Dolap

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4/12/95

Invented by

Record d by

Duan

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4/12/95

Project No. _____

Book No. _____ TITLE _____

From Page No. _____

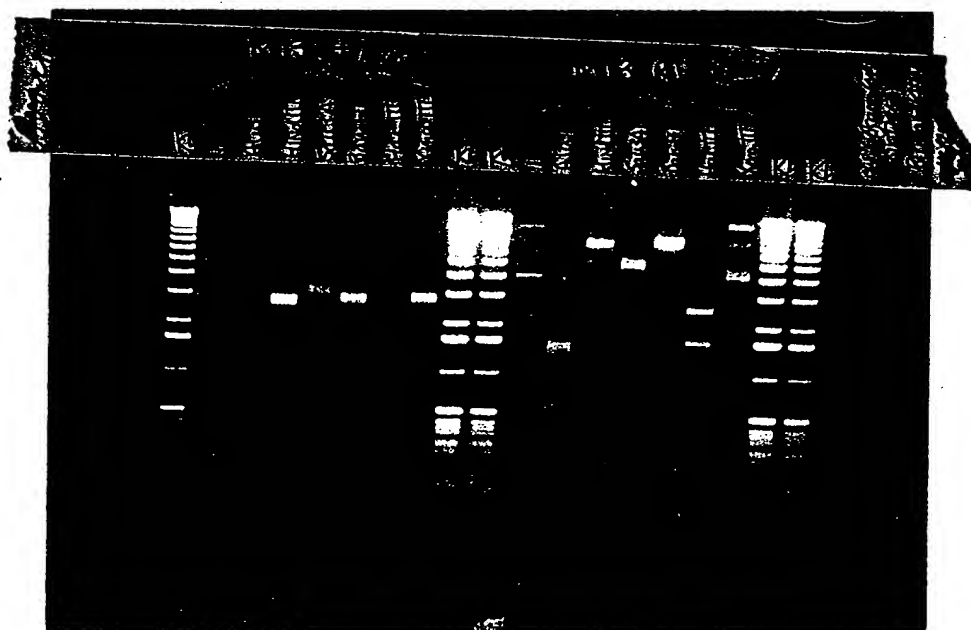
Tube# 1 T-neal / PTTG

1.0 ml

Tube# 2 T-neal / PTTG

1.0 ml

- Cfg. for 1 min. at room temperature
- discarded supernate and added: 100 μ l SI to the pellet. mixed
200 μ l S2 lysis put both tub
ice.
- Cfg for 5 min. at 4°C
- transferred 400 μ l of supernatant to the new tubes.
- added 800.0 μ l EtOH to the supernatant
- put both tubes in the fridge till tomorrow (2/16/95)



exp 4/12/95

(+) SALL3AT - gel shift (did not cut but binded)

To Page No. _____

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4/12/95

Invested by

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4/12/95

Project No. _____

112

Book No. _____

TITLE _____

From Page No. _____

Purpose: Since results are inconclusive -dV (new) (control gave non specific smear with Deepvent - previous (page 110) and gave the same type of smear with old + dV primers attempted to see whether this smear can be transformed into bands!

altered few conditions : checked 2 dif amounts of template

↓
according to NCB suggestions:

1. reduce amount of Template
2. " " of cycles
3. increase Mg
4. " dNTP

200 & 100 pg

tried first 3.

20 + 30 cycles

2, 4 + 6 mM

= also included as controls were : Tag + (Tag + dV)

tried at 200 pg, 30 cycles, 200 μM dNTP with 2, 4, 6 mM

Two sets of reactions were made one with old dV p and the other with new - dV primers

Added Cocktail with different enzymes + later added more Mg accordingly. Used 10x Deepvent buffer has 2 mM Mg already

except for the Tag + (Tag + dV) control rest were run duplicates.

used 1:0.01 mix

94°, 3'

20" (94°, 30")

30" (58°, 30")

72°, 3' /

Deepvent used 1 unit / reaction

20 / x

To Page 1

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12/16/94

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Date

11/30/94

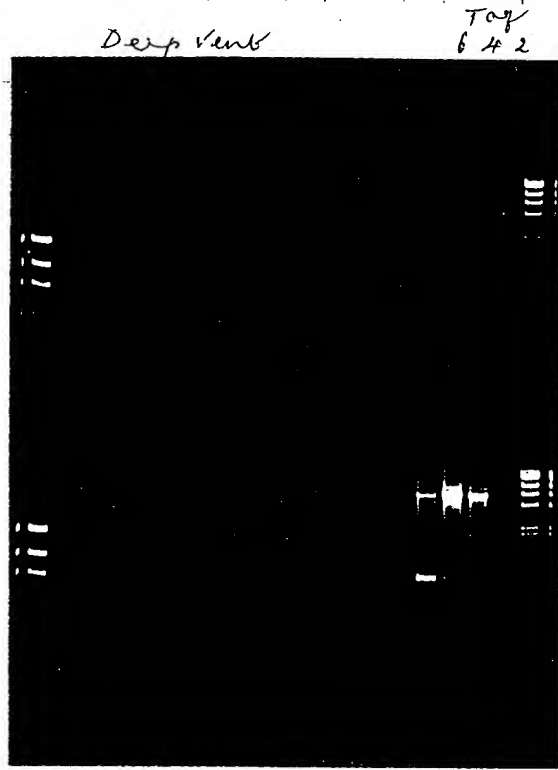
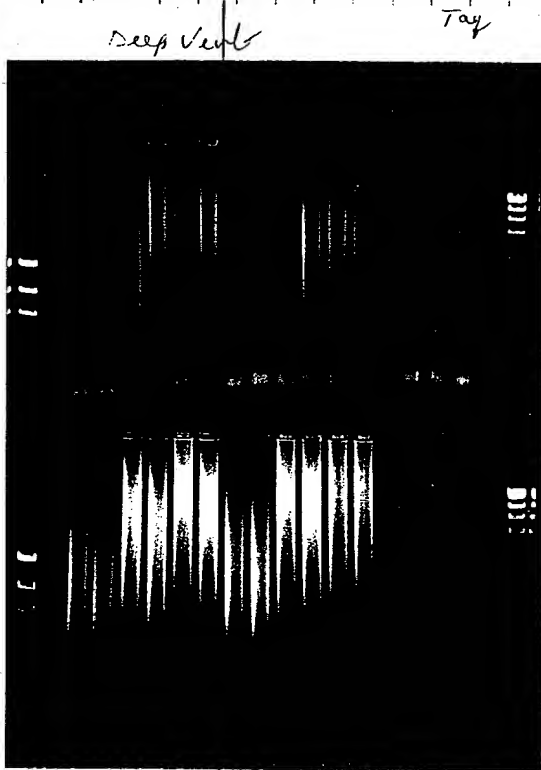
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Project No. _____
Book No. _____

113

age No. new du primer Act II old dc primer 2725 8 2729



Pg 200 100 642 200 100
2 4 1 2 4 6 Tag + DV

with Deep Vent
these primers
give readers

nothing worked

Consistent

- with Tag + DV
works
- w. Tag alone
it doesn't

with Tag alone and
Tag + Deep Vent
nothing not even
readers' result
reproduction

at 50° annealing

New primers are no good.

To Page No. _____

sed & Understood by me,

[Signature]

Date

12/18/64

Invented by

Recorded by

ck. Stannan

Date

11/30/64

76

Project No. _____

Book No. _____

TITLE

Digestion of 5'P Z3mer by Vent
± Tfl, Cheng vs Vent buffer.

From Page No. _____

3'P Z3 mer (P75)

(1) (2) (3) (4) (5) (6) (7) (8) (9)

3 3 3 3

Also with
ribo a
5' mer

2'P Z391

3

P 2692

3

P 2696

3

P 2698

3

P 2700

3

10X Vent buffer

10

10

10

X

✓

5X 67M Cheng

20 20

X

Mig (OAc) 12mm

7.5 7.5

X

0.9ml
+ 0.3ml
(cf: 1.2)

Vent 0.1u/l

1

Tfl 1u/l

1.24

1.24

H₂O

86

85

68.5

70

86

X

✓

100 µl

70°C

remove 10 µl to 5 µl appt. seq. stop solution at

2, 5, 10, 20, 60 for (1)-(4)

(will 1 is Z3mer 0 time)

and

0, 3, 10, 60 for (5)-(9)

(take 0 point before add)

Program 143 = 70°C ∞

To Page No

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Deena a Polarp

Date

11/24/94

Invented by

Date

11-2-94

Recorded by

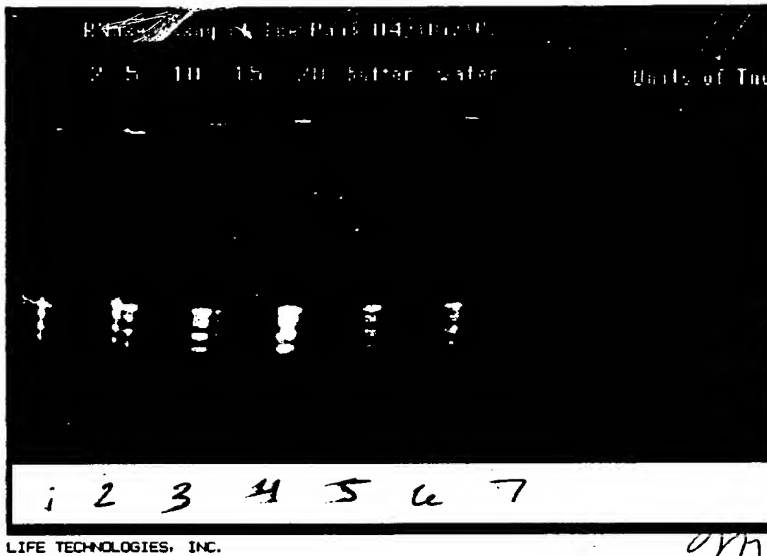
Project No. _____
Book No. _____

^{g-micro}
TITLE Completion of RNase Assay -

From Page No. _____

Take samples from -20°C freezer - spin in micro centrifuge
15 minutes -

decant etOH - air dry pellets -
Add - m of RNA blue juice - heat 30 sec at 90°C
Run out on 7.6%
sequencing gel -
400 volts -

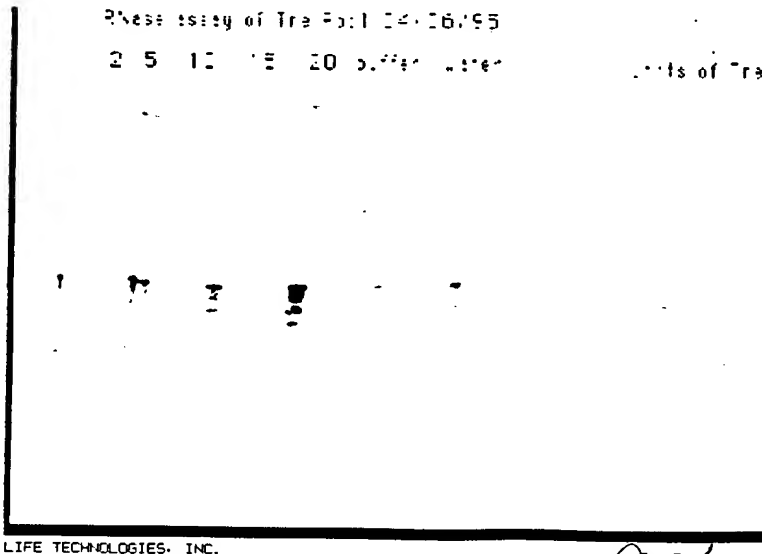


24-04/06/95

Conclusion -
Appears to be
RNase free! Next
time use more RNase -
Only used half of
recommended amount
used 1ug vs recommended
2ug.

Bradford on Pools

24-04/06/95



24-04/06/95

24-04/06/95

To Page N

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May Longo

Date.

4/13/95

Invented by

E. Flynn

Recorded by

Date

04/06/95

Exonuclease Assay - Tne Pool

Project No. _____

Book No. _____

123

Page No. _____

c. NO. 30042 SOP.

| Tube | Rxn mix
4.5 | Enzyme Units | μ | H ₂ O |
|------|----------------|--------------|-------|----------------------|
| 1 | ↓ | 0 | - | 5 μ |
| 2 | | 2.0 | ← | 4 μ 50 μ |
| 3 | | 5 | 1 | |
| 4 | | 10 | 2 | |
| 5 | | 15 | 3 | |
| 6 | | 20 | 4 | |
| 7 | | 0 | | 5 μ dil'n buffer |

Rxn Mix

16 rxns -

10x PCR

80

50mM MgCl₂

80

S' ds sub

16 pmol 32 μ 5 pmol/ μ

B' ds sub

16 pmol 32 μ 5 pmol/ μ

H₂O

494

720

Heat @ 37°C for 1 hour - 1-7

Heat @ 72°C for 1 hour - 8-14

see page - 124 for data

To Page No. 124

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Date

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Date

Man tonge

4/13/95

R c rded by

04/06/95

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Book No. _____

TITLE Endo Assay - 18038 QCP-T

From Page No. _____

Rxn Mixture - in 8 rxns -

(all tubes once before use -)

15264-013
 .34 µg/µl
 10x PCR buffer - 40 µl ✓
 50 mM MgCl₂ - 40 µl ✓
 → ΦX174 (± DNA) - 8 µg (23.5 µl) ✓
 Autoclaved H₂O 256.5

360 µl

Endo mix

H₂O

Diluted enzyme 50/u

| | Endo mix | H ₂ O | Diluted enzyme 50/u |
|---|----------|------------------|---------------------|
| 1 | 45 | 5 | |
| 2 | 45 | 1 | 2 units - 2 µl |
| 3 | 45 | 4 | 5 units - 1 µl |
| 4 | 45 | 3 | 10 units 2 µl |
| 5 | 45 | 2 | 15 units 3 µl |
| 6 | 45 | 1 | 20 units 4 µl |
| 7 | 45 | 5 Dil'n Buffer ✓ | |

Incubate @ 72°C in 3 hours -

90F

37°C

5.5 hours

Tag

Double Strand Assay -

25460-027
 EF 102 1702
 .33 µg/µl
 10x PCR buffer 40 ✓
 50 mM MgCl₂ 40 ✓
 - ΦX174 RF 8 24.2 ✓
 Autoclaved H₂O 255.8

360 -

| | Endo | H ₂ O | Dil. Enzyme 50/u |
|---|------|------------------|------------------|
| 1 | 45 | 5 | |
| 2 | 45 | 1 | 2 4 µl 50/u |
| 3 | 45 | 4 | 5 1 µl |
| 4 | 45 | 3 | 10 2 µl |
| 5 | 45 | 2 | 15 3 µl |
| 6 | 45 | 1 | 20 4 µl |
| 7 | 45 | 5 Dil'n buffer ✓ | |

To Page 1

With ss d & Understood by me,

Date

Invented by

Dat

Man Longo

4/13/95

R cord d by

04-06-95

Endo Assay -

Project No. _____

Book No. _____

125

Page N. _____

Spin samples down add 5ul of Blue Juice -
Run out on 1.2% Agarose gel -

1 2 3 4 5 6 7 8 9 10 11 12 13 14



1 2 3 4 5 6 7 8 9 10
H₂O 2 5 10 15 20 B

8 9 10 11 12 13 14
H₂O 2 5 10 15 20 B

C = 4.0
1.0

at 10u -

4.5
1.0
4.5
conv
5.1%
10.1%

SS-Endo

DS-Endo

Endo looks good - however DS Endo - shows conversion to linear but this is also present in the buffer only lane - could just be a contaminant in the Dil'n buffer -

Dil'n Buffer used - from A.G. (taken from the 4°C Deep cooler - orange tip -

Conclusion: - free of SS Endo nuclease - possible ^{some} DS endo nuclease but control w/tn buffer only shows significant conversion to linear so believe this is the dil'n buffer in or has ^{DS endo} activity not the enzyme prep.

To Page No. _____

Assessed & Understood by m ,

Date

Inv nted by

S. Figure

Date

5/04/95

Recorded by

Mary Tonga

4/13/95

Project N _____
B ok N _____

23

The mutant Phe to Ala

- ge No. _____
- ① The same phenyl alanine corresponding to Tag polymerase will be changed to tyrosine
 - ② For exo D will be changed to Alanine (corresponding region of Tag).

Brian cloned the SphI fragment of Tne Pol into M13mp.

I isolated the single stranded DNA from CJ236 as described before in Bio rad manual.

Test 5µl ssDNA

The DNA looks real good.

For D-A (3'-5' exo mutant oligo) is
5' GA | CGT | TTC | AAG | CAC | TAG | GGC | AAA | AGA # 2899
Eco47III site

For Phe → Tyr (O-helix) HpaI.
GTA | TAT | TAT | AGA | GTA | GTT | AAC | CAT | CTC | TCC | A
2904.

kinased 2899 before.

kinased 2904 as follows:

2µl oligo (210 pmol)
6µl 5X buffer (350mM Tris pH7.6, 50mM MgCl₂, 50mM KCl, 5mM PME)
1µl 10mM ATP
0.5µl T4 Kinase (5U)
20.5µl H₂O
5 (at 37°C) → Heat at 65°C + 3µl TE.
To Page 10.22

sed & Understood by m ,

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4/8/95

Invented by

Recorded by

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Date

3/14/95

Project No. _____
Book No. _____ TITLE _____

114 11/30/94

From Page No. _____

Prayers: Amplify GAPDH for ~~analysis~~ - cloning and ~~transformation~~ in different enzymes again.

- GAPDH / Regular Forward & Reverse primers / DeepVents worked (pg 97)
- never tried with Tag + DeepVent,
- but no problem with Tag alone (pg 88).

used: DeepVent buffer - 2 mM Mg
200 μM dNTP
0.15 μM primer
100 pg template (10 pg/μl)
25 (94°, 30", 60°, 1' 15"
72°, 10

| Tag: | | | Unit | | | Tag + DeepVent | | | DeepVent | | |
|------|-----|-----|------|-----|-----------|----------------|----|--|----------|--|--|
| # | | | # | | | # | | | # | | |
| 1 | 2 | 0 | 31 | 32 | 10 + .001 | 15 | 16 | | | | |
| 3 | 4 | .5 | 33 | 34 | .005 | 17 | 18 | | | | |
| 5 | 6 | 1 | 35 | 36 | .01 | 19 | 20 | | | | |
| 7 | 8 | 1.5 | 37 | 38 | .05 | 21 | 22 | | | | |
| 9 | 10 | 2 | 39 | 40 | .1 | 23 | 24 | | | | |
| 11 | 12 | 2.5 | 41 | 42 | .2 | 25 | 26 | | | | |
| 13 | 14 | 5 | 43 | 44 | .5 | 27 | 28 | | | | |
| 15 | | | 45 | 46 | 1 | 47 | 48 | | | | |
| | 15x | | | 17x | | | | | | | |

10x buffer 75
dNTP 15

Template 150
primer 1 3.75
2 3.75

50 μl / Rx

Cocktail → 50 μl / Rx

enzyme diff. amount
D.V + 10 Tag in all

20x

50 μl
+
diff. am
2
deep vent

added enzyme
separately in 1 μl

To Page

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Invented by

Date

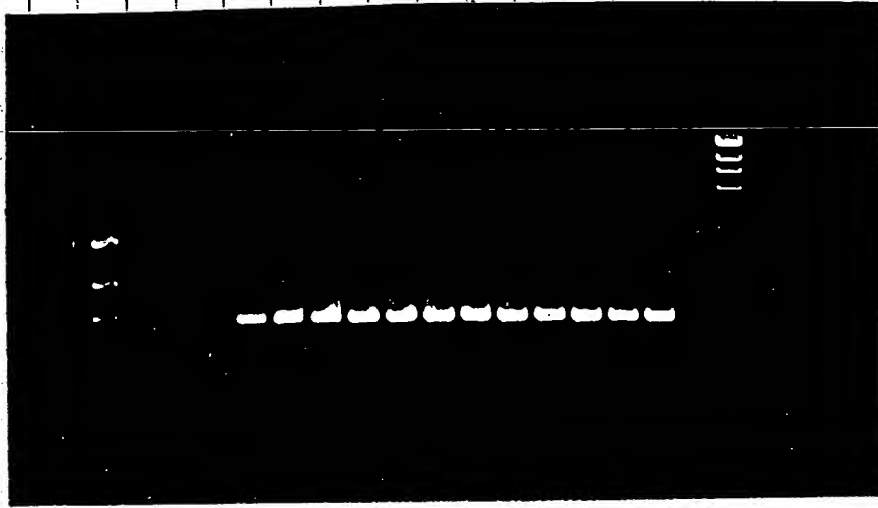
Recorded by

11/30/94

K. Sturman

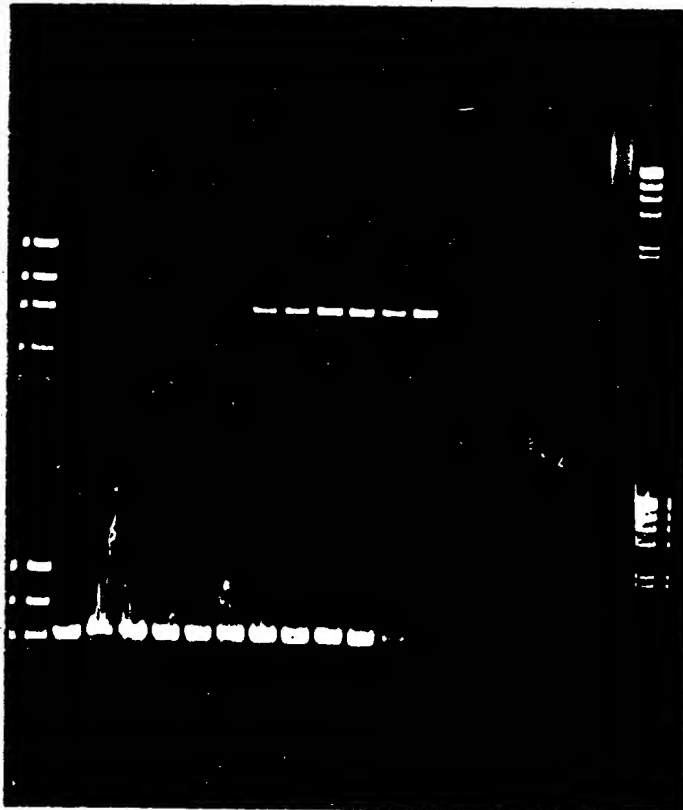
ag No. _____

Tag



0 .5 1 1.5 2 2.5 5

.001 .005 .01 .05 .1 .2 .5 1 2



10 Tag .001 .005 .01 .05 .1 .2 .5 10 Deepvent

Result:

- 100 pg + 25 cycles seem to be enough
- Deepvent alone with regular primer worked again
- Deepvent at lower concentration works better. with 0.05 U good product yield was seen.
- So may be with earlier run, if the enzyme conc. is lower, it might have worked with Deepvent. Try?

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Date

12/11/94

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K. S. Araman

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12/11/94

To Page No. _____

Project N _____
Book N _____

regressing with Tne

Page No. _____

32P23m (075)

5.3

✓

PUC18 RF

10.5

✓

10x Tag buffer

~~3.5~~ 4.5

✓

H₂O

14.5

✓

Tne 2 μ l

1

✓

dilute in Tag storage buff

36 μ l

8.5 8.5 8.5 8.5

ddA

2

C

2

G

2

T

2

To Page No. _____

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11/29/94

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11-2-94

Project No. _____

Book N . _____

TITLE _____

78

From Pag No. _____

cycle reg.
the pool
ACCTACCT

Vent buffer Cheng Buffer

HF1

min

0 2 5 10 20 60

2 5 10 20 60

2 5 10 20 60

2 5 10 20 60

-- -r S- SR - S

10 3 10 60 10 3 10 60 10 3 10 60 10 3 10 60



2/11/94 3 211-10,000
Saucy

To Pag

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Date

11/29/94

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R c rded by

Date

11-2-94

Tue. 8-5 8x minus.

3-5 210
Tue 3/30/95

Page No. _____

Track BFR (7.1) - Suspended 11.5 grams in 30mls A-8FR

50mm Tris - 7.5 - 100ml
0.5mm EDTA - 2ml
0.2mm PMSF - 4ml
8.1g/glycine - 100ml
5mm Bml - 6.99ml
10mm kcl - 6.7ml

Sandata w/med. TIP - output - 8-
3x 1min -
1:200 w/dtts ^{ED} 595
Crude = 0.74
#3 = 0.17

Heat kill 85°C for 10 min
Cool - 0 Make 50mm kcl (phosphate conc.)
② ADD 0.4% P.E.I.
→ spin in 55-57 15min - 20G's -
- Decant Sup 3.3 mls
60% Nky 50% cut = 390g/l = 12.5

→ 1.75mg/ml Resin
Equilibrate 8ml B50 Hydrophobic Column w/1: - 8ml = 3.44 x 0.25 =
8ml = 0.785 = 10.2 cm


2- 2.5mm Tris - 7.4
8.1g/glycine -
0.5mm EDTA
10mm kcl
5mm Bml
0.1mm PMSF.

Pressurized pellet 1:1 w/dtts -
- Dialyzed in A- vs. 20 vol's -
- 12.5T gradient = 96 mls
1.6 ml/fraction x 60 fractions

Same (+) 1.5ml kcl.
gradient = 1.75 ml/mils.
55 min =

To Page N _____

| | | | |
|---|-----------------|--|-----------------|
| sed & Understood by me,

May Long | Date
5/31/95 | Invented by
 | Date
5-22-95 |
| | R corded by | | |

Proj ct No. _____
Bo k No. _____

13

ag N _____

2/21/95 TUE

DIGEST T.nea/pSPORT with SstI & SphI

DIGEST M13 mp18 & M13 mp19 w/ SstI & SphI

M13 mp18 RF (0.44 ug/ul) } cut 500.0 ng
M13 mp19 RF (350.0 ug/ml) }

$$\rightarrow 1000 \text{ ng/ug} \times 0.44 \text{ ug} = 440 \text{ ng}$$

$$\frac{500 \text{ ng}}{440 \text{ ng}} = 1.1 \text{ ul}$$

$$\rightarrow 1000 \times 0.350 \text{ ug/ul} = 350 \text{ ng}$$

$$\frac{500 \text{ ng}}{350} = 1.4 \text{ ul}$$

mp18

mp19

| | |
|---------------------------------|----------------------------|
| H ₂ O - 35.0 ul | H ₂ O - 35.0 ul |
| 10x buffer - 2.0 ul ← REact 2 → | 10x buffer - 2.0 ul |
| 500ng DNA - 1.1 ul | DNA - 1.4 ul |
| 1ul SstI - 2.0 ul | SstI - 2.0 ul |
| 40.0 ul | 40.0 ul |

T.nea/pSPORT

H₂O - 81.0 ul
10x buffer - 10.0 ul
ng/ul DNA - 4.0 ul
SstI - 5.0 ul
100.0 ul

- Incubated all 3 tubes @ 37°C for 1/2 hour
- Made 0.8% agarose gel
250.0 ml TE buffer
2.0 g Agarose
- boiled for 4.0 min.
- added 12.0 ul E. Bromide
- poured the gel.

To Page No. _____

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SD okup

Project No. _____

Book No. _____ TITLE _____

14

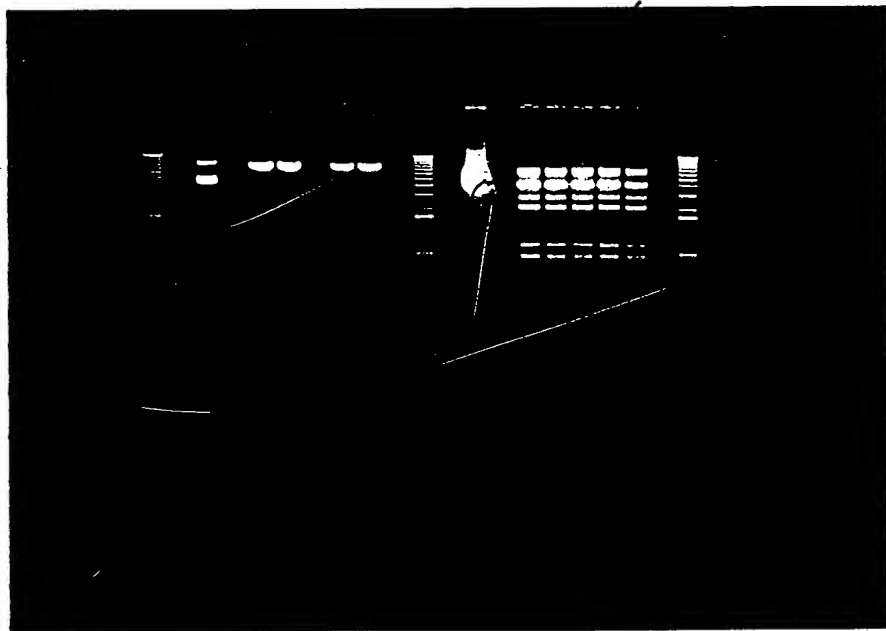
From Page No. _____

added: 2.0 μ l of 1M KCl 2 μ g = 2000 ng
to 40.0 μ g (mp 18 & 19)

5.0 μ l of 1M KCl
to 100.0 μ g (pSPORT)

added ~~Sph~~ Sph I - 2.0 μ l mp 18
2.0 μ l mp 19
5.0 μ l pSPORT

- Incubated @ 37°C for 1/2 hour
- put the tubes in the fridge till
- ran samples on the gel ~~also~~ on 2/22/95



arp 2/22/95 ①

M13mp18 & mp19 RF D
are ds, supercoiled forms
the DNAs of phages M13
& 19. Using this vector
foreign DNA can be
inserted into the mul
cloning site in an
oriented fashion.

T Pag N

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Date

4/12/95

116

12/1/94

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

Purpose : - To try new primers again with pM09
- to get rid of mispriming optimization of Mg.

used : KlenTag buffer w/o Mg added Mg later, different conc.
5 µl.

| | | | | | | |
|-----------------|--------------------|----|-----------|------|----|------|
| 1 Unit tag | | | | | | |
| 1 µM primer | included | mm | 1 | 1.5 | 2 | 2.5 |
| 200 µg template | primer 1 alone | | 5 | 7.5 | 10 | 12.5 |
| 200 µM dNTP | 2 " | | 45 | 42.5 | 40 | 37.5 |
| | w/o primer | | 50 | | | |
| | w/o Mg as controls | | 1 | | | |
| | | | 5 µl / rx | | | |

Cycling : 94° 3' 30 (94° 30", 56° 30", 72° 3')
72° 30"
4°

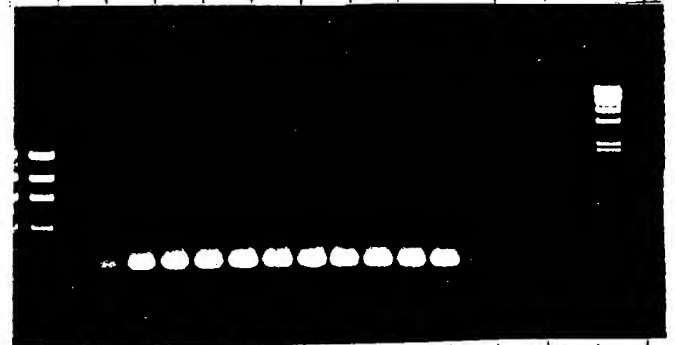
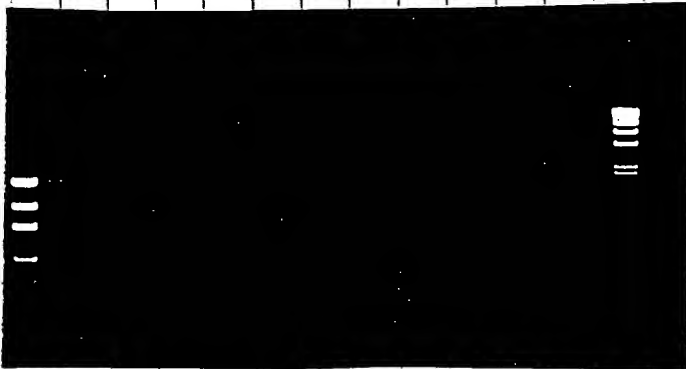
| | |
|------------------|-------|
| 10 x buffer | 60 |
| dNTP | 120 |
| Template | 2.4 |
| enzyme | 2.4 |
| primer 1 | 6.0 |
| 2 | 6.0 |
| H ₂ O | 451.2 |
| | ↓ |
| 45 µl + 5 µl | Mg |

- Did the same with new
dV primers.

2728
2729

10.7 + 10.6 primers 1 & 2
1441.9 H₂O

w/o primers assembled separately



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To Page

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Book No. _____

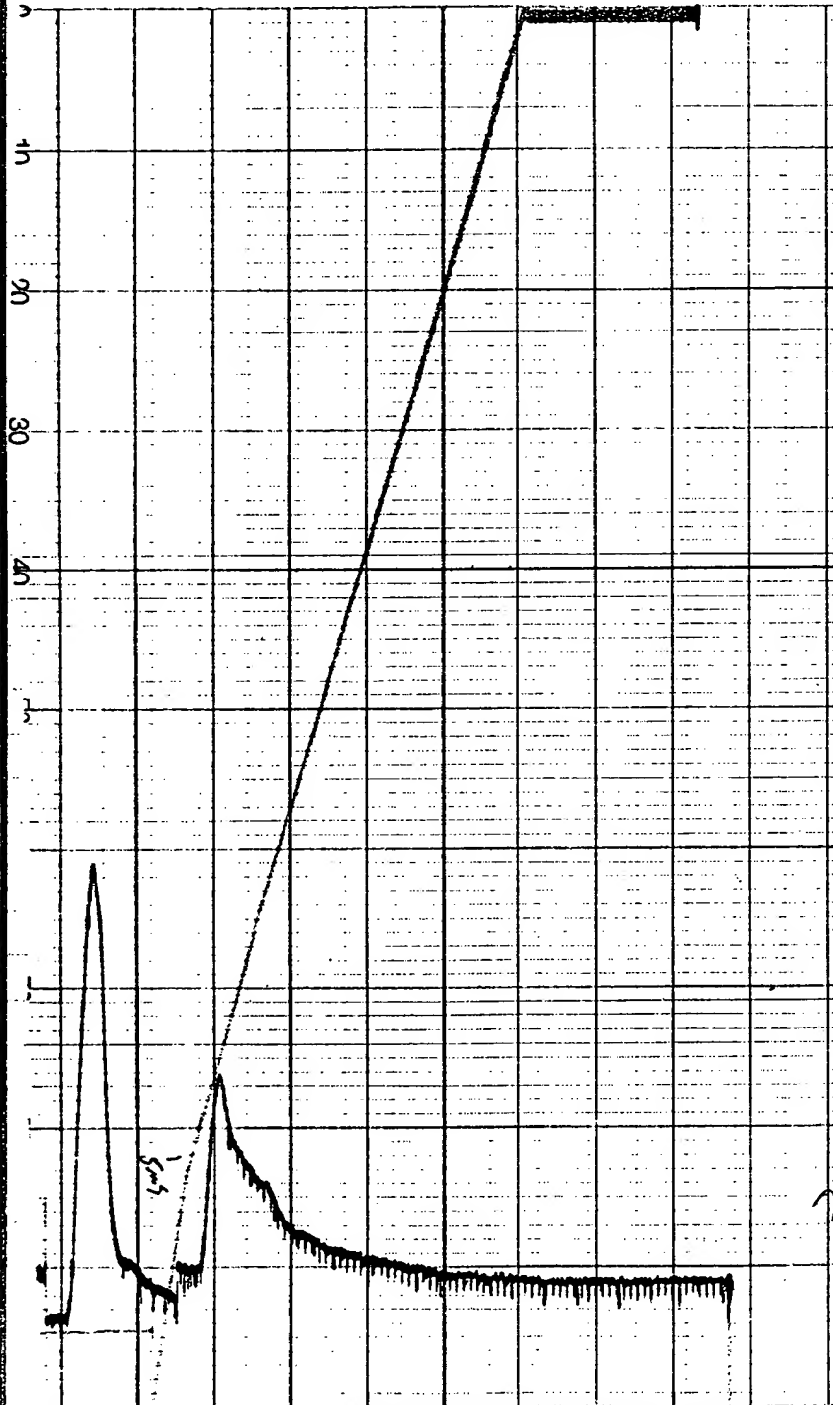
TITLE

TR50 HEP 650 m -
Repeat of Ind Assay - Enzyme

130

From Page No. _____

8ml elution -
Gross column -



Curve
Post H.D.
Am 503-549

| | | |
|------|----|--------|
| 20AD | | |
| WT | 1 | 892.00 |
| WT | 2 | 440.00 |
| 4 | 3 | 198.00 |
| 6 | 4 | 80.00 |
| 8 | 5 | 74.00 |
| 10 | 6 | 72.00 |
| 12 | 7 | 70.00 |
| 14 | 8 | 64.00 |
| 16 | 9 | 92.00 |
| 18 | 10 | 82.00 |
| 20 | 11 | 62.00 |
| 22 | 12 | 86.00 |
| 24 | 13 | 74.00 |
| 26 | 14 | 58.00 |
| 28 | 15 | 102.00 |
| 30 | 16 | 58.00 |
| 32 | 17 | 58.00 |
| 34 | 18 | 96.00 |
| 36 | 19 | 48.00 |
| 38 | 20 | 96.00 |
| 40 | 21 | 80.00 |
| 42 | 22 | 64.00 |
| 44 | 23 | 66.00 |
| 46 | 24 | 100.00 |
| 48 | 25 | 64.00 |
| 50 | 26 | 102.00 |

MY
5/31/95

MY
5/31/95

Pharmacia LKB Biotechnology

Code No. 18-1001-44

To Page

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Amey Lomzo

Dat

5/31/95

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5-31-95

age No. _____

- Sow mix 2x - dilute #3 -
 - Fungal stain 2x - dry ice -

Eg mix - A =

25 mm Tiz - 7.4

81 g/gal

O. 1 ml in EDTA

20 mm kel

5 mm at Base

- Heat kill 85°C - 10 min -

- Assay pre + post H₂O treatment

1 104.00

2 150.00

3 104.00

4 128.00

5 9012.00

6 146.00

7 114568.00

= Same (f) 1.2 m/kel

To Page No. _____

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Date

Invented by *A*

Date

*May Jongo**5/31/95*

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5-2595

80

Project No. _____
Book No. _____

TITLE 23mer degradation: V, UV, Inc
buffers: Cheng vs Vent vs. KlenTag

From Page No. _____

| | ① | ② | ③ | ④ | ⑤ | ⑥ | ⑦ | ⑧ | ⑨ | ⑩ | ⑪ |
|---------------------------|------|---|---|--------|------|------|----|---|---|----|---|
| Cheng buffer 5X | 20 | → | | | | | | | | | |
| 10x KlenTag buffer * | | | | 10 | → | | | | | | |
| Vent buffer | | | | | | | 10 | → | | | |
| Toz storage buffer | | | | 2 | 2 | - | | | | | |
| Mg OAc 12 mM | 9.5 | → | | | | | | | | | |
| Mg SO ₄ 100 mM | | | | 1.2 μl | → | | | | | | |
| glycerol 50% | | | | | | | | | | 16 | → |
| DMSO 100% | | | | | | | | | | | |
| 32P 23mer ** | 3 μl | → | | | | | | | | | |
| Vent pol 0.05 μl | 2 | | | 2 | | | 2 | | | 2 | |
| Deep Vent 0.05 μl | | 2 | | | 2 | | | 2 | | | 2 |
| Tne 0.5 μl | | | 2 | | | 2 | | | 2 | | |
| H ₂ O | 6.55 | → | | 81.8 | 81.8 | 83.8 | 85 | → | | 69 | → |

Preheat to 70°C, start by addition of DNA pol
remove 10 μl to 5 μl cycle reg stop mix at 10, 20, 30 min
well #1 is 23mer uncut
Vp = 100 μl

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Deena Pokany

Date

11/29/94

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11-4-94

To Page

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(14) (15)

✓
✓
✓

✓ ← (note KlenTag requires reagents on Tag storage buffer for glycerol and
Tweens/NP40 - for Tne it is diluted in Tag storage buffer
so no supplement is needed for Vent
and Deep Vent dilution
is in storage buffer
(with Triton and
50% glycerol)

✓ (1.2 mM Mg OAc Cf)

✓ (1.2 mM MgSO₄ Cf)

✓ Cf = 8% glycerol

✓ Cf = 2% DMSO

✓

} dilute in Vent/Deep Vent storage/dilution buffer (its
2.1% Triton)
(dilute in Tag storage buffer)
0% Cf = 0.002%
Triton
will include
2 µl Tag storage
buffer next time
(similar to TFL
storage buffer with
0.5% Tween/NP40)

✓

2 µl, 0.66 pmol
3 µl, 0.66 pmol
12.0 µl, 0.66 pmol
13.5 µl (8.91 pmol)
16.8 µl (2.5 pmol)
55 µl
0.36 pmol primer

** for 72p23 mix 0.66 pmol/1 13.5 µl
plus 16.8 µl cold 5'3' 23 mix plus
24.7 µl H₂O. 0% Vf = 55 µl and specific
activity is reduced 2x

* 10x KlenTag is 500 mM Tris HCl pH 9.0
160 mM (NH₄)₂SO₄ and no MgSO₄

To Page No. _____

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Dat

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maria Polanco

11/29/94

R cord d by

11-4-94

1g N

2/22/95

1. grow cells overnight (O/N) 10.0 mL
= 9.0 mL (1.0 mL in ea. nine tubes). Each tubes labelled AH10B
P12C17.nca
2/22/95 BJS
LB + AP100
• Quick freeze all nine tubes in a powdered
dry ice.
- GENE CLEAN
- 2) Did electrophoresis of yesterday's DNA (2/21/95)
M13 mp18 and M13 mp19 and pSPORT
- 3) Took the picture of the gel
- 4) cut off mp18 fragment, mp19 fragment & pSPORT fragment from
the gel & transformed the gel w/ ^{pa} DNA into the separate
appendix tubes.
- 5) added 100.0 μ L NaI to each ² tubes. Vortexed mp18 & mp19 tubes.
- 6) Incubated both tubes @ 55°C to melt agarose. mixed after incubation
- 7) added 5.0 μ L glass milk to both tubes.
- 8) Incubated both tubes on ice for 5 min.
- 9) ctf. both tubes (quick spin)
- 10) discarded supernate
- 11) added 500.0 μ L New Wash buffer
- 12) discarded supernate & again added 500.0 μ L New Wash buffer.
washed both tubes 3 times.
- 13) added 10.0 μ L dH₂O to the tubes. mixed well by vortexing. 55°C for 2-5 min
- m) set up Ligation

| | |
|--|---|
| H ₂ O = 12.0 μ L
ligase 5x Buffer = 4.0 μ L
mp18 DNA = 2.0 μ L
(1.0 μ L) Ligase = 2.0 μ L
TV = 20.0 μ L | H ₂ O = 12.0 μ L
5x buffer = 4.0 μ L
mp19 DNA = 2.0 μ L
Ligase = 2.0 μ L
TV = 20.0 μ L |
|--|---|

- n) Incubated both tubes overnight @ room temperature (cond)

To Page No. _____

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4/12/95

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(con'd)

T.nea/Ptnc E. pttc

- 1.0 mL of ea.
- cfg.
- discarded supernate
- added 100.0 μ l SI mixed well
- Incubated on ice for few min.
- added 200.0 μ l S2 lysis
- Incubated on ice for few min.
- added 150.0 μ l S3 w/ RNAase A
- cfg. for 7.0 min @ 4°C

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Project No. _____

Book No. _____

TITLE _____

118

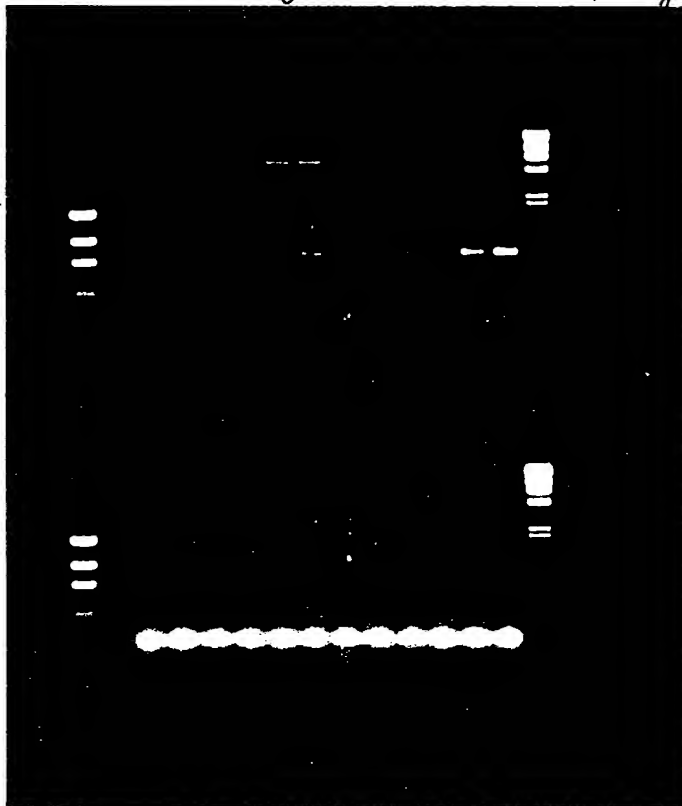
12/1/94

From Page No. _____

Purpose: Since 1U of Tag - titration with Mg didn't work, increase the amount of enzyme to 2U.

- Both new + old dV primers were tried.
- Expt was done under same condition as I.

old dV 0 1.5 2.5 2.5 2.5 3.0 mM Mg



new dV

Result:

- mispriming persists
- 2U better than 1U of
- 2U / 1.5 mM Mg ✓
- again new dV for didn't work - can give up.

* Try again with new dV primers + increasing amount Tag + Mg.

To Page 1

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120

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

- used Max efficiency DH5α ~~α~~ RK3 101 to transform
- control puc 5 μl = 50 pg } + 100 μl of Competent
- 5 μl of each U3G R^x }
- add 900 μl of ^{50c}competent cells and let
to express for 1 hr.
- plated control - diluted 1:10 20, 50, 100 μl
- Test - unv " 20, 50 & 100 μl
- 100 μl from each plated x 2
- Agouti counted them all.
- Amp - depletion resulting in
large satellite colonies - to
be score.
- According to Agouti in Mr treated the counts were 50 -
- Rest not much difference between balanced & input into
reaction, ~~was~~ still more white than (normal) red
line of mine.
- 10 fold increase in the present set of plates
= 10% white.
- plated a few more from each reaction for better
accurate count. These plates were treated with 50 pg
of fresh Ampicillin.
- prepared in total of 100 μl.

12/6/94

To Page 1

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Date 12/6/94

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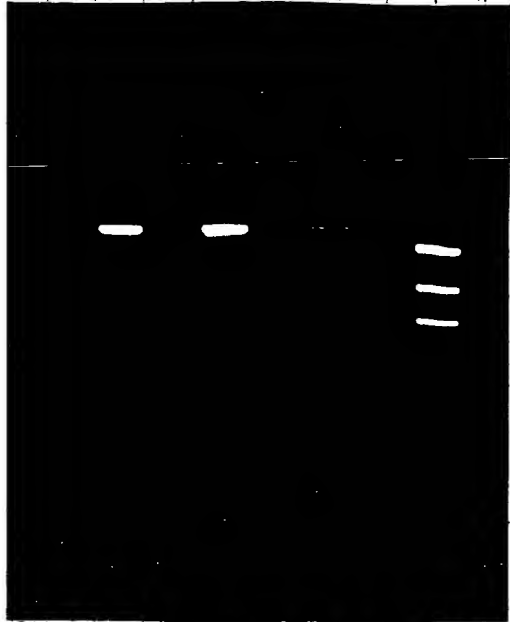
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Date

12/6/94

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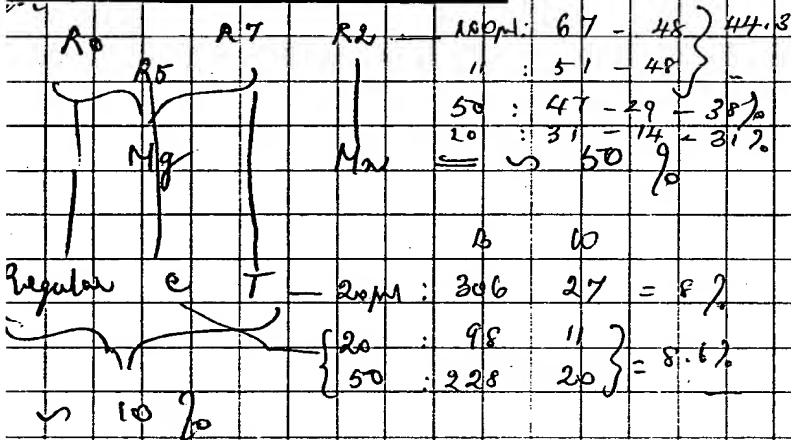
50 ng
30
20
10
5
2.5

- agent did another reaction with Tag, different cycle # and 2 dif. con. of target template, 5 pg and 50 pg w. the du primers & pvc.

- % cycles: 10, 20, 35 & 40

- with 10 cycles no product in both con. of target

with 20 cycles, 5 pg target barely visible product 50 pg gave a faint amount which was quite visible.



both 5 & 50 pg, quite a good amount of product yield with 35 & 40 cycles. With 50 pg more than 5 pg & 40 cycles more than 35 cycles as expected.

Did transformation of all the products. more amp spread out 500 µl plate.

All with Tag.

Result: colony & too low even at 35 & 40 cycles. But high % of colonies unexpectedly high even at lower # of cycles??

np: no problem - even in BPP plates satellite colonies with Ax & not with control - something to do with test reactions + transformation
colony yield did not correspond w. # of colonies obtained?
correlation between # of cycles and # of errors??

To Page N _____

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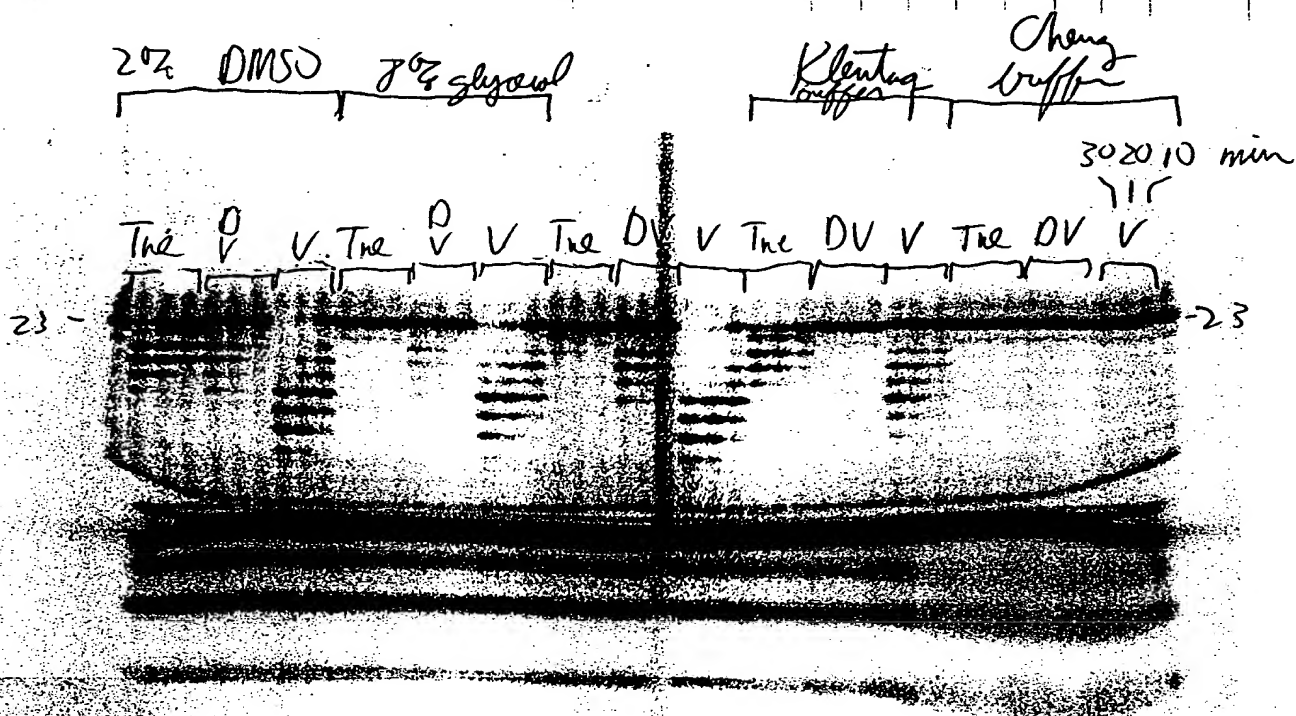
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12/7/94

Dr. Silasamun

From Page No. _____



Result.

To Page

Witnessed & Understood by me,

Deena Polay

Date

11/29/94

Invented by

Recorded by

Date

11/5-94

From Page No.____

| | Chemical | KlenTag
buffer | DMSO | 7% glycerol | 20%
glycerol |
|---|------------------|-------------------|------|-------------|-----------------|
| Inhibitor pH 8.7 | Trio-Hill pH 9.1 | | | | |
| K ⁺ DAc ⁻ pH 8.7 | | | | | |
| K ⁺ el | | | | | |
| (NH ₄) ₂ SO ₄ | | | | | |
| Mg(OAc) ₂ | | | | | |
| Mg SO ₄ | | | | | |
| DMSO | | | | | |
| Inhibitor | | | | | |
| Tween-20/NP40 | | | | | |
| glycerol | | | | | |
| same mixture | | | | | |
| Vault | | | | | |
| Deep vault | | | | | |
| Tue | | | | | |

To Page N

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Deena Golay

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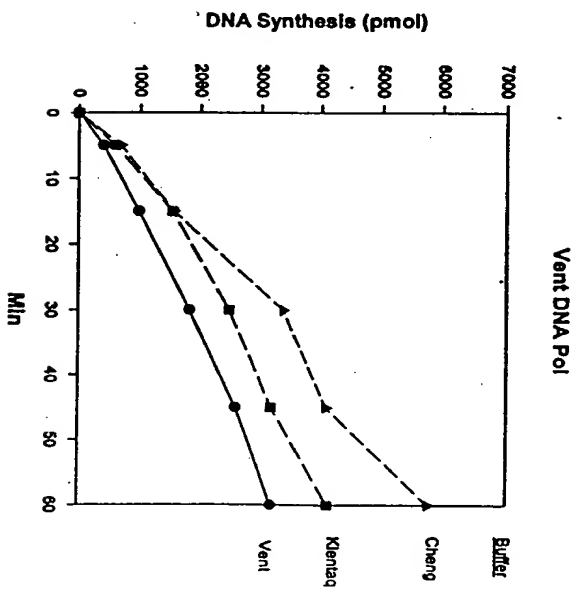
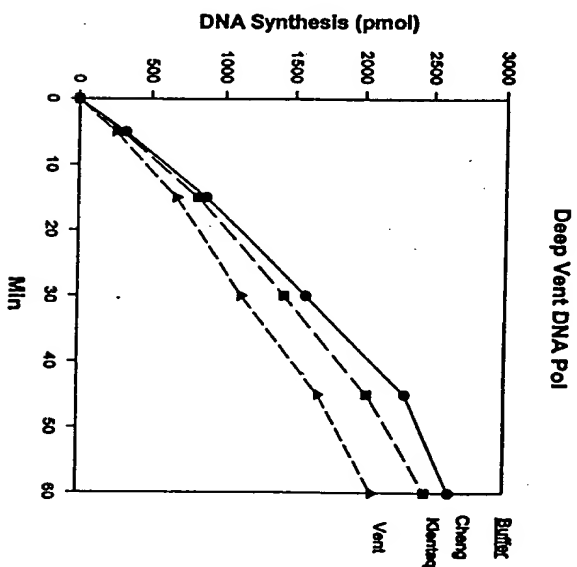
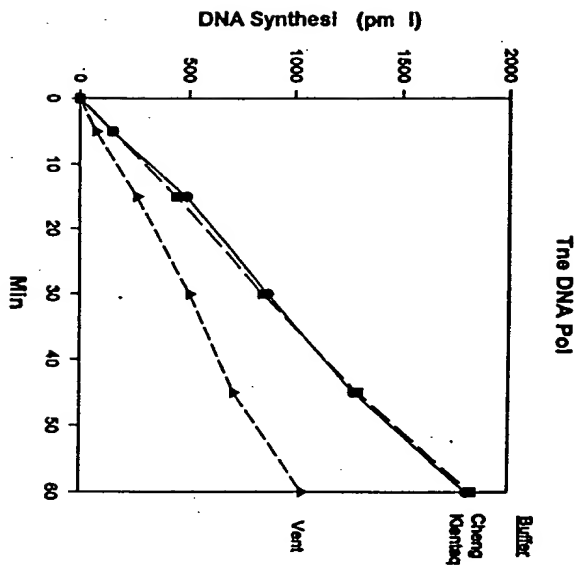
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Date

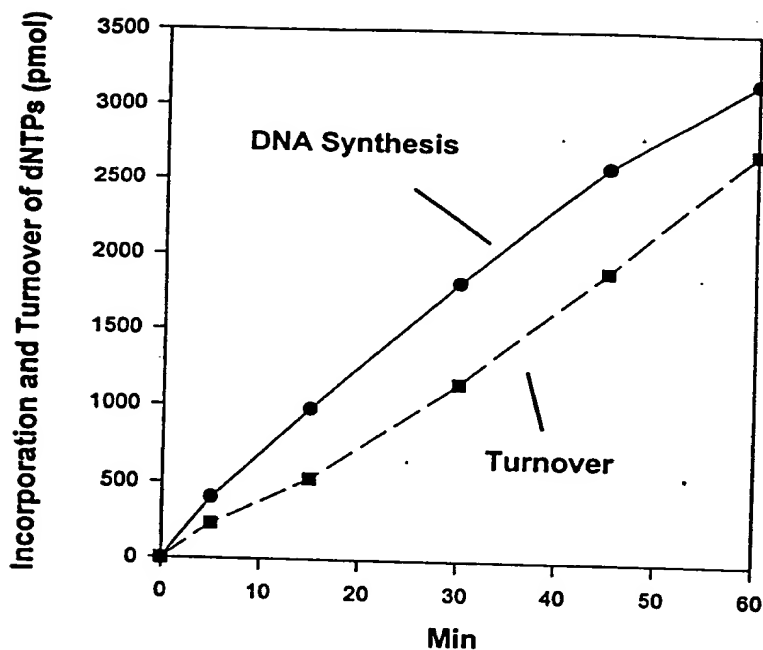
115-94



In each case, DNA synthesis is lower in
 Primer degradation was highest in Vent

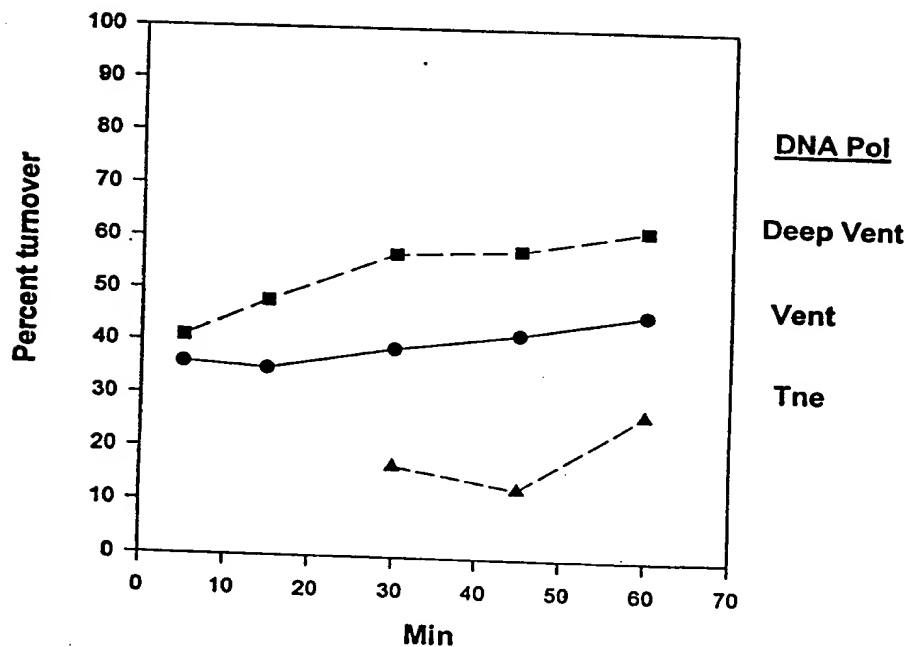
got Turnover
 by DNA synthesis
 1, below

Vent DNA Pol in Vent Buffer



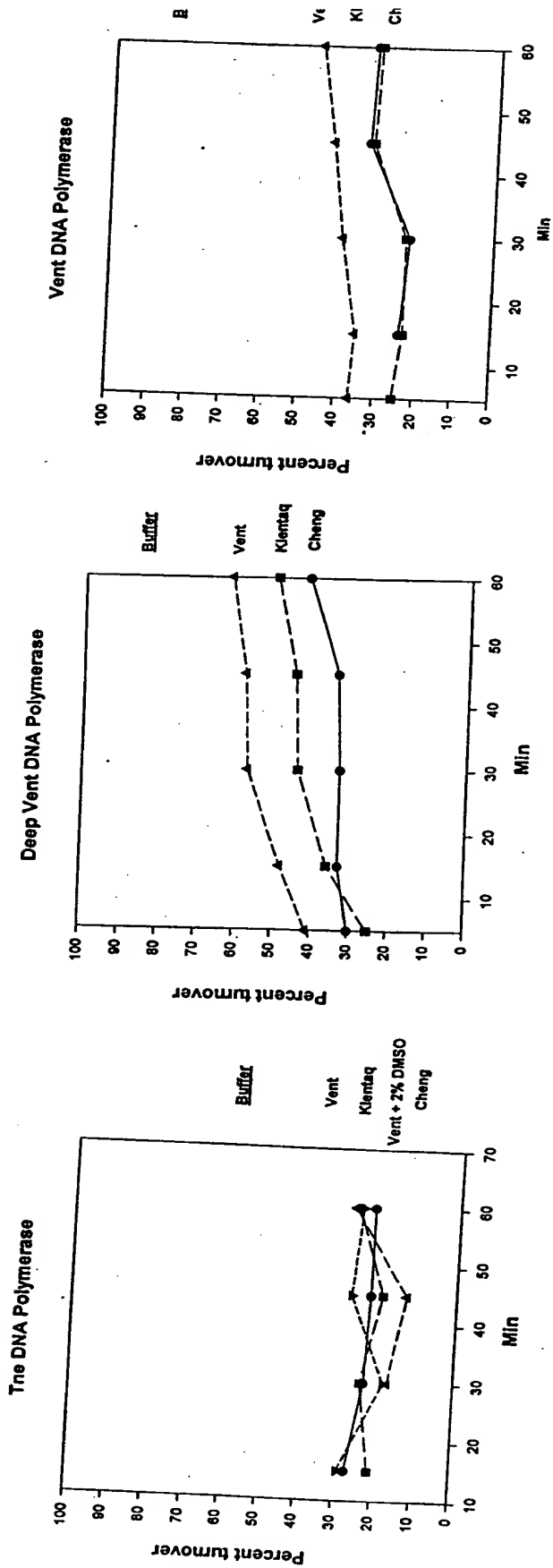
DNA synthesis
and turnover
to dNMP

Activity in Vent Buffer



Percent turnover
turnover
incorporation + turnover

Deep Vent has
higher turnover
than Vent as
expected. Tne
is ~2x lower
than Vent and
Deep Vent



effect of buffer on turnover is not large compared to effect on primer degradation

ssed & Und rstood by m ,
erica Polcup

Date
11/29/94

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11-5-94

Project No. _____

Book No. _____

TITLE New 3'-5' exo nuclease Mutant of T₄

From Page No. _____

4/13 -

Purpose: ~~See~~ Previous clone (P. 129) of a 3'-5' exonuclease mutant of *Thermotoga neopolitana* (Tne) proved not to ~~produce~~ over express a heat sensitive or no polymerase activity. ~~Here Gre Del's~~ ~~predecessor~~ made a new clone. The purpose of his experiment is to screen pre + post heat kill for a polymerase activity. If activity is ~~more~~ thermostable then proceed w/ a PET + $(NH_4)_2SO_4$ ppt.

3 grams of cells - suspend in 1 ml of crack buffer -
sonicate with ~~mini tip~~ micro tip ~~ultrasonicator~~

crack buffer -

20 mM Tris pH 7.5

10 mM KCl

1 mM EDTA

5 mM Bme

5% glycerol

#575 - .824 before crack

A575 - .198 after crack

76% crack -

6 x 20 sec
micro tip C
setting 4.Save 400 μ l \rightarrow No heat treatment

Aliquot the rest of the cracked material to 2 ml eppendorf
heat kill 10 min \rightarrow temp. @ 80° - 90° C -
note: temperature rose to > 90 for maybe up to 5 min

Spin in microfuge @ 14000 g 30 minutes -

heat treat supernatant < 90° > 85° C - for 5 minutes -
spin in microfuge @ 14000 x g 10 minutes -

To Page 1

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May 20/95

Date

6/20/95

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6/13/95

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06/13/95

say in thermostable polymerase activity -

mix - TAD premix - premade by A.G. -

add 1.1 μ l / 500 μ l premix
of 2320 dCTPDilution $5/495 - \frac{10}{90} - \frac{1}{1000}$
 $290 - \frac{1}{3000}$ $1/1000$ 1 μ l
2 μ l heat treated $1/3000$ 1 μ l
2 μ l $1/1000$ 1 μ l
2 μ l before heat treatment $1/3000$ 1 μ l
2 μ l mistake made - put 78 μ l of premix should have only used 48 !!incubate 10' @ 74°C in a heated water block - quench rxn w/ 10 μ l of 5M EDTA - spot 30 μ l on 6H/C 11.2i

wash filters

1x 10% TCA 5'

3x 5% TCA 3'

2x 5 to H

dry + count in econofluor LSF

AM CPM1

1 $1/1000$ 2994.002 $1/1000$ 2384.003 $1/1000$ 622.004 $1/1000$ 888.005 $1/1000$ 3612.006 $1/1000$ 5296.007 $1/1000$ 1234.008 $1/1000$ 1662.009 $1/1000$ 964.0010 $1/1000$ 90094.0011 $1/1000$ 89736.0012 $1/1000$ 89120.0013 $1/1000$ 40.00

H Kill

No H Kill

S.A

27 06/13/95

First approx. - looks as though not as much activity after heat & kill - need to do less dilutions to in order to ascertain what exactly is going on.

Seeing most polymerase act. in No Heat Kill?

Repeat w/ $1/500$
 $1/200$ dil's.

mz

0/20/95

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is d & Understood by me,

Date

Invented by

Date

Recorded by

Nay Tonyo

0/24/95

Elizabeth Hyman

06/13/95

Project No. _____

Book No. _____

TITLE _____

20

From Page No. _____

2/28/95 TUE

I set up digest DNA ppt.

① M13mp 18, ② mp 19 and ③ T. nea / pSPORT

1. - To ea 3 added 100.0 μ l TE } to ppt
" " 10.00 μ l NaAc } DNA
" " 300.00 μ l EtOH }

2. Incubated on dry ice for ~5 min.

3. Cfg. for 10 min. @ room temp. (no ppt) &

4. no ppt., added 2.0 μ l (carrier molecule) Yeast tRNA. Vortexed

5. incubated on dry ice for ~5 min.

6. Cfg for 10 min. @ room temp. (Supernate saved) Pellet was saved on mp 18 &

7. added 200.0 μ l 70% EtOH to the pellet

8. Cfg. discarded supernate, air dried by putting tubes on the heat block.

II DIGEST set-up. (to map Bam HI site)

- Cut T. nea / pSPORT with Hind III, Bam HI, Xba, NOT I, Sst, EcoR
Separate

H₂O - 13.0 μ l

buffer - 2.0 μ l

T. nea / pSPORT DNA - 3.0 μ l.

enzyme - 2.0 μ l

TV = 20.0 μ l

Enzymes - Hind III, Xba, Sst had REact 2 1
buffer.

- Bam HI, NOT I, EcoRI had REact:
buffer.

Control: H₂O - 13.0 μ l

(REact2) buffer - 2.0 μ l

DNA - 3.0 μ l

for
separate
enzymes.

- Incubated @ 37°C

- ran on the gel on 3/1/95 (Wed)

Picture shown pg 21

To Page N

Witness d & Underst od by me,

Date

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Dat

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4/12/95

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4/12/95

ag N _____

I DIGEST set-up

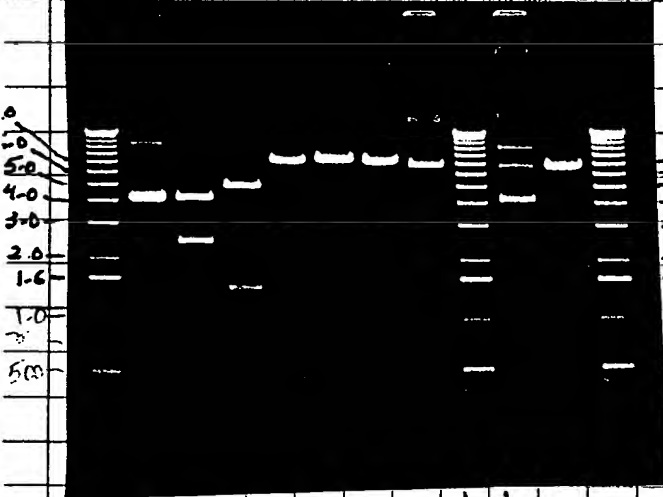
- Cut ptna / T. nea w/ Sst I enzyme

H₂O - 11.0 μ l
 d2) buffer - 2.0 μ l.
 T. nea) DNA - 2.0 μ l.
 Sst I - 5.0 μ l.
 TV = 20.0 μ l.

Control: H₂O - 11.0 μ l
 buffer - 2.0 μ l.
 DNA - 2.0 μ l

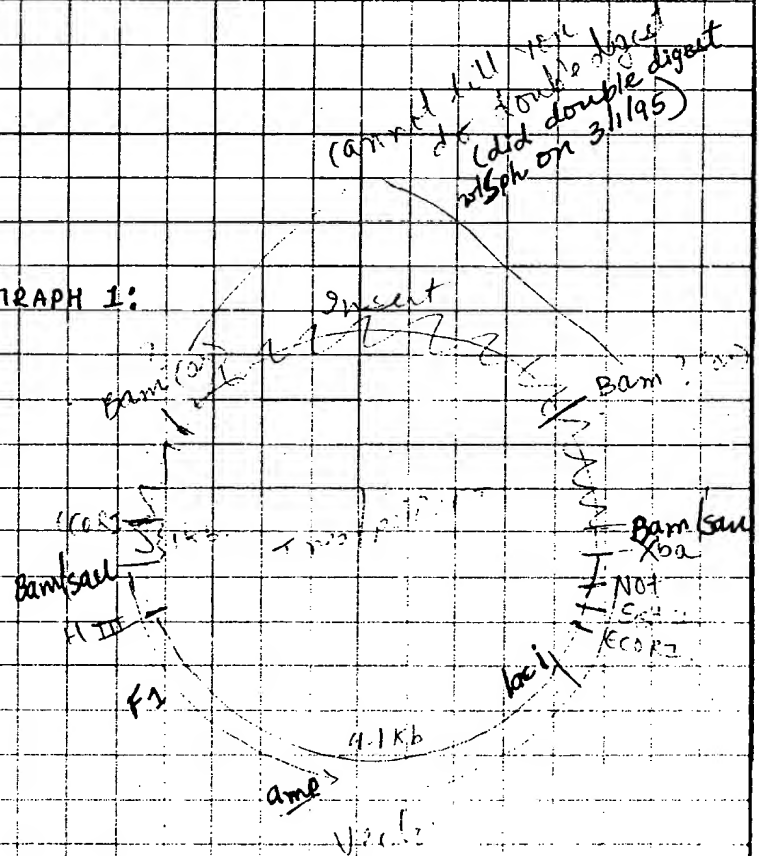
- Incubate @ 37°C

- Lam DNA on a gel on 3/1/95 (wed) picture shown below



from pg 20

: GRAPH 1:



: GRAPH 2: pg 23 of this book.

To Page No. _____

s d & Understood by me,

Date

4/12/95

Invented by

Recorded by

Date

4/12/95

Project No. _____

Book No. _____

TITLE

Turnover for Vent, Deep Vent, -
(follow P. 61, 7)

B4

From Page No. _____

| | | | |
|----------------------------|----------------|-----------|------|
| | (A) | (B) | (C) |
| H ₂ O | 399 | 487 | 489 |
| 5x Chelex buffer | 133 | 4.67 | 476 |
| 10x KlenTag | | 66.7 | |
| 10x Vent buffer | | | 66.7 |
| Tag storage buffer | 6.71 | | |
| 3.7 mg/ml activated DNA | 90 | | |
| JATG-TTP 10mM each | 3.33 | | |
| 32P dATP 10mCi/ml | 1.02 λ | | |
| Mg(OAc) ₂ 50 mM | 1.6 μ l | | |
| MgSO ₄ 100 mM | | 8 μ l | |
| DM50 10.0 μ l | | | |

| | | | | | | | | | |
|------------------------|----------|-----|-----|-------|-----|-----|-------------------|-----|-----|
| | 0.055 ml | | | 0.633 | | | 2.633.650 use 180 | | |
| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) |
| | 195 | 195 | 195 | 180 | 190 | 150 | 190 | 190 | 19 |
| Tag storage buffer | 4 | | | 4 | 4 | - | 4 | | |
| Vent 0.08 μ l | | 4 | | | 4 | | | 4 | |
| Deep Vent 0.08 μ l | | | | | | 4 | | | 4 |
| Taq 0.07 μ l | | | 4 | | | | | | 4 |

primers to 70°C, start by addition of pol 5 6 6

remove 15 μ l to 5 μ l 0.2 M EDTA \rightarrow spot 15 μ l on GE
and remove 5 μ l to 5 μ l Kill solution (20 μ mol/l DAPI
100 mM EDTA) at 2

0 5 15 30 45 60 min
spot 2 μ l on PEI resolve in 1M LiCl

* dilutions of pol
name as P.81

Results: see graph on P.81

T Page N.

Witnessed & Understood by me,

Deena Bokay

Date

11/29/94

Invented by

Recorded by

Date

11-9-94

g N (1) 14.4 ✓
 ✓
 ✓
 66.7 20 ✓
 → 27 ✓
 ✓
 → 1 ✓ (Cp = 50 μm each)
 → 0.36 ✓ (220 x 10⁶ total cpm)
 ✓ (1.2 mM Mg(OAc)₂)
 ✓ (1.2 mM MgSO₄ in Klenow buffer)
 4 μl ✓ Cp = 2% DMSO (2 mM MgSO₄ in 1X Vent buffer)
 (10)
 19.4 ✓ (0.4 units total of each pol)
 4
 1

⇒ Cf = 0.005% Tween 20/NP40
 so this makes up for no TPE
 here - its present in Joes
 long PCR Rxn.

| | | | |
|---|------------------|--------------------------------|-----------------|
| I & Understood by me,
<i>nae a Polak</i> | Date
11/29/94 | Invented by
<i>R. Polak</i> | Date
11-9-94 |
| | | R corded by | |

Project No. _____

Book No. _____

TITLE _____

134

From Page No. _____

48 μ l of premix aliquoted to pre labeled appendants 4/1

| | | | |
|---|-------|---|------------------|
| 1 | 1/200 | 1 | } post Heat Kill |
| 2 | | 2 | |
| 3 | 1/500 | 1 | |
| 4 | | 2 | |

| | | | |
|---|-------|---|-----------------|
| 5 | 1/500 | 1 | } pre Heat Kill |
| 6 | | 2 | |
| 7 | 1/500 | 1 | |
| 8 | | 2 | |

incubate @ 74°C in a heated water block - for 10 minutes
quench w/ 10 μ l of 5M EDTA - spot 30 μ l on GPC

Wash 1x 10% TCA 1x Pi 5'
3x 5% TCA
2x EtOH

dry + count w/ Econofluor

USER: 2 ID: 32P
SAMPLE REPEAT:
H#: 1 AQC: N QCF
CHANNEL 1-LL:
DATA CALC: CPM.
HALF LIFE (DAYS):

| SAM | CPM1 |
|---------|----------|
| 1/200 | 8460.00 |
| 2 | 21680.00 |
| 1/500 | 8296.00 |
| 4 | 7486.00 |
| 1/200 | 16274.00 |
| 6 | 28614.00 |
| 1/500 | 8412.00 |
| 8 | 17912.00 |
| 9 | 2794.00 |
| 10 S.A. | 91294.00 |

| | |
|------------------|-------|
| } post heat Kill | 11.9 |
| | 17.98 |
| | 29.9 |
| | 28.4 |
| } pre heat Kill | 30.1 |
| | 29.54 |
| | 39.7 |
| | |

S.A. 57 cpm/pmol nt

mf
6/20/95

To Page 1

Witnessed & Understood by me,

Date

Invented by

Dat

May Jones

6/20/95

R c rded by

[Signature]

06/16/95

'ag No.____

27. SDS PAGE - 3'-5' exo minus the



5/10/10 L

124 6/20/55

1 2 3 4 5 6 7 8 9 10
1 3 5 μ 5 μ 10 μ 20 μ 1 M
pre heat post heat
kill kill

u heat - @ 20 $\mu\text{g}/\text{ml}$ - spun down sup loaded on gel
st heat @ .36 $\mu\text{g}/\text{ml}$

To Page No. _____

ssed & Understood by m ,

Date

Invented by**Date**

May Longo

6/20/95

Recorded by

3.4 gm

06/16/95

Project No. _____

Book No. _____

TITLE

#41 - S. Sgm crack

From Page No. _____

Purpose: to screen FYI - 1 mutant Tne - one base point mutation phenylalanine to Tyrosine 1 for thermostable polymerase activity.

S. Sgams cells - resuspend in 10 mL of crack buffer - p. 132 ~15

Divide in two 7.5 mL samples in 15 mL conical
Sonicate w/ microtip @ max output - 5
6 x 20 sec bursts

Before - A₅₉₅ .750 ~ 57% crack
After - A₅₉₅ .320

Sonicate again - 3 x 20 sec bursts

Before A₅₉₅ .750 ~ 73% crack
After A₅₉₅ .200

Divide Save 400 mL - pre crack material
Aliquot remainder of crack into 2 mL eppendorf
incubate @ 87°C 11 minutes -
Spin in microfuge 20 minutes @ 14,000

Decant and save supernatant → assay for
thermostable polymerase activity -

To 500 μ L of TAD pre mix add 1.1 μ L of ^{p32} dCTP

48 μ L of pre mix / rxn - 1.2 μ L of
diluted samples added incubate @
74°C in a heated water block -
for 10'. Quench rxn w/ 10 μ L of

5M EDTA - spot 30 μ L of GFC
Wash 1x w/ 10% TCA 1x w/ 1% Pi @ 5'
3x w/ 5% TIA @ 3'
1x w/ EDTA

dry + count -

| | | |
|---|-------|---|
| 1 | 1/200 | 1 |
| 2 | | 2 |
| 3 | 1/400 | 1 |
| 4 | | 2 |
| 5 | 1/200 | 1 |
| 6 | | 2 |
| 7 | 1/400 | 1 |
| 8 | | 2 |

To Page N

Witnessed & Understood by m ,

Date

Investigated by

Date

Recorded by

Mary Longo

6/20/95

E. Flynn

6/16/95

Page N _____

USER: 2 ID:32P
SAMPLE REPEAT:
H#: 1 ADC:N BCF
CHANNEL 1-LL:
DATA CALC: CPM
HALF LIFE(DAYS)

Note: Background very high!

06/14

Debr note: Did not induce for very long -

| SAM | CPM1 |
|-----|----------|
| 1 | 5722.00 |
| 2 | 7676.00 |
| 3 | 2608.00 |
| 4 | 4686.00 |
| 5 | 10454.00 |
| 6 | 19114.00 |
| 7 | 5850.00 |
| 8 | 11594.00 |
| 9 | 2976.00 |
| 10 | 90102.00 |
| 11 | 88418.00 |

Post Heat Kill 5.8 5.4 U/ul
5.0
Pre Heat Kill 15.9 U/ul 17.1 U/ul 17.0 U/ul
18.3

loss of 70%?

any 6/20/95 Appears to have (very high background) + lost activity after heat kill & maybe mostly host cell activity in pre heat kill?

Bradford's

Slope - .0555 O.D./ug

| | | | |
|-----------------------------|------|---------|-------------------|
| Heat Kill Tyl - 40 | .474 | * too ↑ | .2 ug/ul |
| 20 | .333 | | .289 ug/ul |
| Pre Heat Kill Tyl - . | .259 | | 23.3 ug/ul (19.8) |
| | .439 | | 136 ug/ul |
| Post Heat Kill 3'-5'ero mut | .303 | | |

Run 12x SDS PAGE - see p 138

1-2-3-4
↓

To Page N _____

is d & Understood by me,

Date

Invented by

Date

May Longo

6/20/95

Rec rded by

06/16/95

Project No. _____

Book No. _____

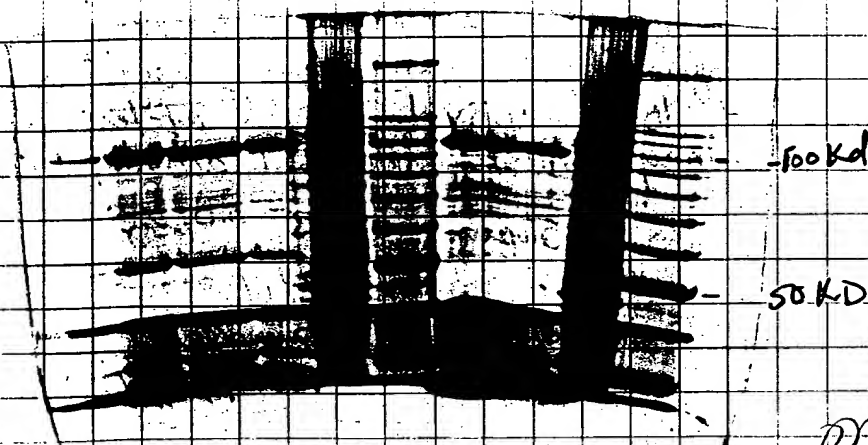
TITLE P41 + 3'-5' exo mutant SDS PAGE

From Page No. _____

06/11

12% SDS PAGE.

1 2 3 4 5 6 7 8 9 10

1 6 μ g 4 μ g 2 μ g 60 μ g M 4 μ g 2 μ g 60 μ g MFYI
post heat
killFYI
pre heat
kill3'-5' exo M.
post heat
killpre heat
kill 3'-5' exo-

pre heat kill - spun down sup loaded on gel

To Page 1

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May Longo

6/20/95

Record d by

06/16/95

Project No. _____

Book No. _____

TITLE _____

22

From Page No. _____

3/2/95

① 1 kb ladder ② T.nra/psport uncut, ③ sst, ④ sst/sph, ⑤ sph, ⑥ 1 kb ladder. (from 263 added loading dye, electrophoresis @ 190 v

- ~~digested~~ double digested BamHI/sphI (to map the Bam site T.nra)

H₂O - 14.0 μ l

control: - H₂O - 14.0 μ l

(REACT) buffer - 2.0 μ l

(uncut) buffer - 2.0 μ l

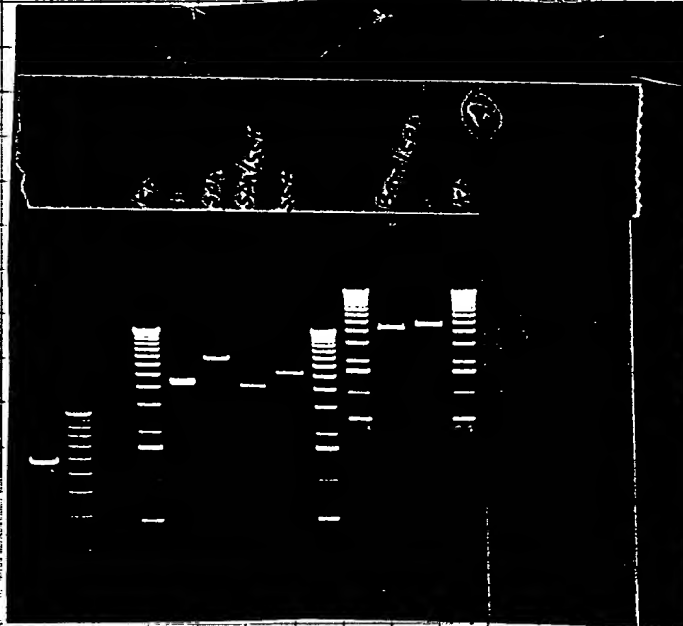
1/10 dil (T.nra/psport) DNA - 2.0 μ l

DNA - 2.0 μ l

(Bam/sph) enzyme - 1.0 μ l ea.

TV = 20.0 μ l.

Incubated @ 37°C for 30 min. (15 min.)



To Page 1

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Date

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Date

[Signature]

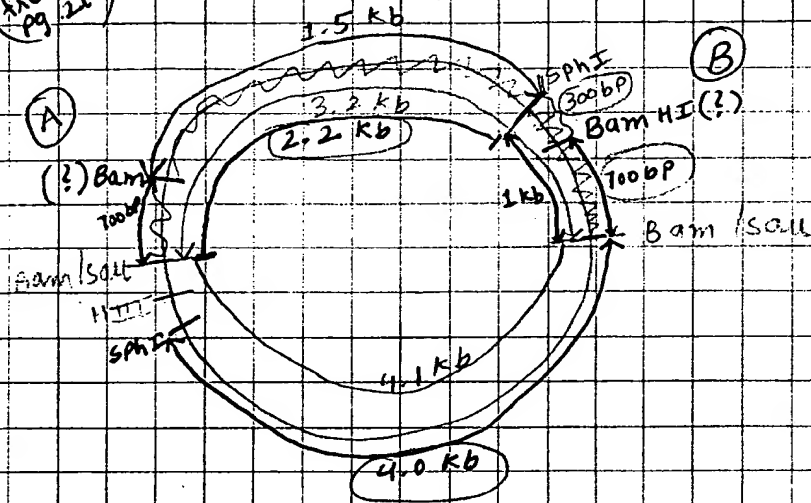
4/12/95

R c r d by *[Signature]*

4/12/95

Page No. (from pg 21)

GRAPH 2:



Bam/Sph

(A)

4.0 kb
700 bp
1 kb
1.5 kb

Bam/SphI

(B)

4.0 kb
2.2 kb
300 bp
700 bp

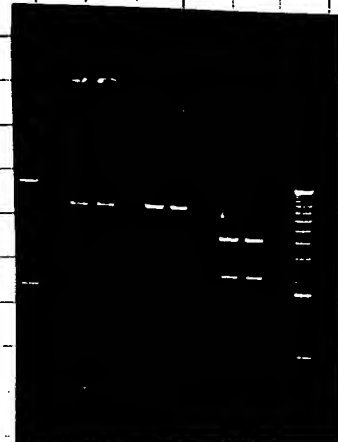
3.2
1.7
1.5

Cloning mp 18 w/ T.na/psport & mp 19 w/ T.na/psport

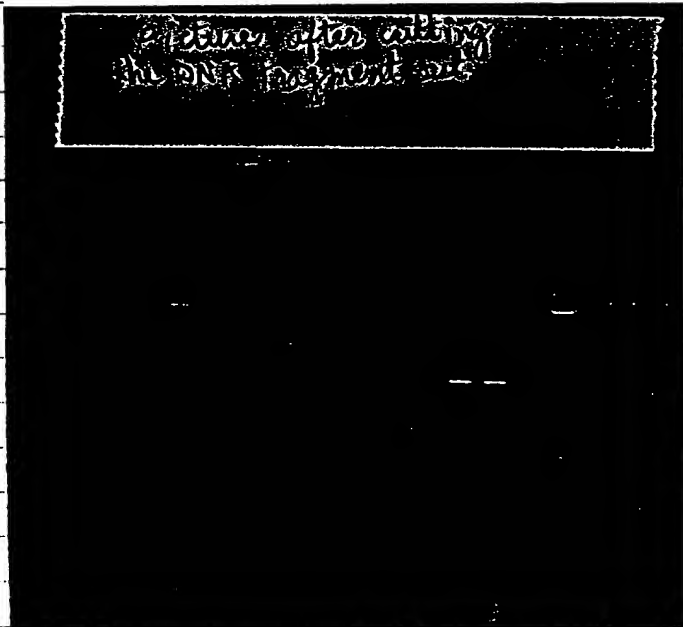
3/2/95

3/2/95

Thurs.



Picture before cutting the DNA fragment.



DID GENE CLEAN.

To Page N _____

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[Signature]

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4/12/95

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[Signature]

Date

4/12/95

Project No. _____
Book No. _____ TITLE PMC9 / Tag / Tag + DV / F+
122 12/6/94

From Page No. _____

Purpose: - To check these primers with 2V of enzy.
+ 3 step cycle.

- Repeat of expt. page 116 - 118 but used

non DV forward & reverse
47.3 μ M 52.9

| 12x of each with Tag or Tag + D.V | | | |
|-----------------------------------|-------|-------|-------------|
| 10x buffer | 499.2 | 499.4 | 200 μ M |
| dNTP | 60 | 60.0 | 200 μ M |
| | 12 | 12.0 | 1 μ M |
| Temp | 2.4 | 2.4 | K.T. buff |
| primer 2 | 11.5 | 11.5 | enzy |
| 1 | 12.7 | 12.7 | Tag + DV |
| enzyme | 4.8 | 12.0 | |

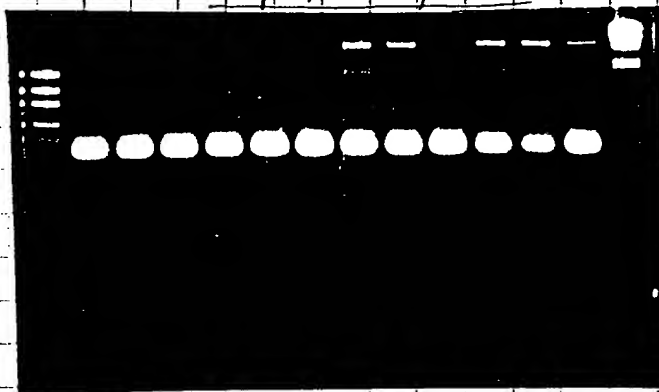
45 μ l of cock tail + 5 μ l of Mg different conc. 1 - 3 mM

Cycles 94°, 3'

30(94°, 30", 56°, 30", 72°, 3')

72°, 10" \rightarrow 4° reheat
Tag + Dup vent

Tag alone samples done



| | | | |
|---|-----------------|-----------------------------------|-----------------|
| With ssed & Understood by m ,
<i>[Signature]</i> | Date
12/6/94 | Invented by
<i>[Signature]</i> | Date
12/6/94 |
| | | Recorded by
<i>[Signature]</i> | |

age No. _____

Results: Tag alone / 3 step cycle / F + R no - dv. didn't work
again.

Tag + D.V. : as usual even with 1V 2 mM - 3 mM coats.

for some reason these new primers at 1 mM con / 50 μ l gives
lot of primer dimers.

Judy's 2 step cycle works with Tag 2V.

Not transformed anything yet.

To Page No. _____

Sed & Understood by m ,

Date

12/19/84

Invented by

Recorded by

Dr. Subramaniam.

Date

12/18/94

| 86 min | | Project No. <u>06</u> | Book No. <u>Turnover</u> | TITLE | Turnover | Ampl |
|--------|---------|-----------------------|--------------------------|-------|----------|----------|
| 01 | 543.00 | BK60 | (14) | | 61 | 269.00 |
| 02 | 650.00 | 110 | | | 62 | 7412.00 |
| 03 | 1014.00 | 476 | 24 | | 63 | 16953.00 |
| 04 | 1485.00 | 771 | 22 | | 64 | 36825.00 |
| 05 | 2627.00 | 2148 | 34 | | 65 | 44610.00 |
| 06 | 3187.00 | 2725 | 32 | | 66 | 62771.00 |
| 07 | 525.00 | BK60 | | | 67 | 241.00 |
| 08 | 662.00 | 141 | 30 | | 68 | 3518.00 |
| 09 | 948.00 | 436 | 33 | | 69 | 9506.00 |
| 10 | 1271.00 | 769 | 33 | | 70 | 17320.00 |
| 11 | 1677.00 | 1188 | 34 | | 71 | 25050.00 |
| 12 | 2340.00 | 1871 | 42 | | 72 | 28643.00 |
| 13 | 624.00 | BK60 | | | 73 | 324.00 |
| 14 | 694.00 | 72 | (32) | | 74 | 1974.00 |
| 15 | 796.00 | 177 | 27 | | 75 | 5340.00 |
| 16 | 880.00 | 264 | 23 | | 76 | 9478.00 |
| 17 | 976.00 | 363 | 22 | | 77 | 13880.00 |
| 18 | 1110.00 | 501 | 22 | | 78 | 19753.00 |
| 19 | 805.00 | BK60 | 785 Ave | | 79 | 321.00 |
| 20 | 977.00 | 192 | 25 | | 80 | 8826.00 |
| 21 | 1409.00 | 467 | 23 | | 81 | 23029.00 |
| 22 | 1803.00 | 762 | 23 | | 82 | 37324.00 |
| 23 | 2832.00 | 1133 | 32 | | 83 | 47661.00 |
| 24 | 3299.00 | 1883 | 31 | | 84 | 61758.00 |
| 25 | 774.00 | BK60 | | | 85 | 404.00 |
| 26 | 918.00 | 99 | 25 | | 86 | 4493.00 |
| 27 | 1406.00 | 415 | 36 | | 87 | 12238.00 |
| 28 | 2277.00 | 1118 | 44 | | 88 | 21497.00 |
| 29 | 2989.00 | 1651 | 45 | | 89 | 30491.00 |
| 30 | 4085.00 | 2472 | 50 | | 90 | 36800.00 |
| 31 | 777.00 | BK60 | | | 91 | 214.00 |
| 32 | 813.00 | 21 | (12) | | 92 | 2257.00 |
| 33 | 947.00 | 121 | 21 | | 93 | 6671.00 |
| 34 | 1136.00 | 263 | 24 | | 94 | 12685.00 |
| 35 | 1204.00 | 314 | 19 | | 95 | 19429.00 |
| 36 | 1631.00 | 633 | 26 | | 96 | 27534.00 |
| 37 | 919.00 | BK60 | 922 Ave | | 97 | 239.00 |
| 38 | 1284.00 | 251 | 36 | | 98 | 7128.00 |
| 39 | 1754.00 | 530 | 35 | | 99 | 17335.00 |
| 40 | 2728.00 | 1150 | 39 | | 100 | 32171.00 |
| 41 | 3910.00 | 1903 | 42 | | 101 | 45795.00 |
| 42 | 5168.00 | 2704 | 46 | | 102 | 56065.00 |
| 43 | 924.00 | BK60 | | | 103 | 318.00 |
| 44 | 1205.00 | 180 | 41 | | 104 | 4474.00 |
| 45 | 1892.00 | 617 | 48 | | 105 | 11839.00 |
| 46 | 3234.00 | 1472 | 57 | | 106 | 19756.00 |
| 47 | 4572.00 | 2325 | 58 | | 107 | 29674.00 |
| 48 | 6365.00 | 3467 | 62 | | 108 | 36540.00 |
| 49 | 863.00 | BK60 | | | 109 | 261.00 |
| 50 | 901.00 | 20 | (7) | | 110 | 1566.00 |
| 51 | 953.00 | 103 | 17 | | 111 | 4647.00 |
| 52 | 1083.00 | 103 | 13 | | 112 | 8879.00 |
| 53 | 1085.00 | 386 | 27 | | 113 | 12496.00 |
| 54 | 1529.00 | 92 | 29 | | 114 | 18327.00 |
| 55 | 984.00 | BK60 | | | 115 | 295.00 |
| 56 | 891.00 | 124 | 18 | | 116 | 1709.00 |
| 57 | 1067.00 | 264 | 34 | | 117 | 4261.00 |
| 58 | 1086.00 | 347 | 25 | | 118 | 8343.00 |
| 59 | 1336.00 | 347 | 25 | | 119 | 12504.00 |
| 60 | 1467.00 | 347 | 25 | | 120 | 18443.00 |

Win

Deanna Polanco

Date 11/29/94

Inv nted by

Record d by

11-9-94

Page 1

g N _____

JAMP BK60^g

1. Chery mix = 564 ave
2. Klentay mix = 785
3. Vent mix = 922

spot

Chery

Klentay

Vent

$$75821 \text{ cpm} \left(\frac{50 \mu\text{l Rxn Vol}}{2 \lambda \text{ spotted}} \right) \left(\frac{200}{195} \right) \left(\frac{1}{2500 \mu\text{l}} \right) \left(\frac{1}{4} \right) = 194 \text{ cpm at } \mu\text{mol}$$

$$(267 \text{ cpm} / \mu\text{mol})$$

$$(314 \text{ cpm} / \mu\text{mol})$$

$\mu\text{mol incorp} =$
(200 $\mu\text{l Rxn}$)

$$\frac{\text{cpm}}{\text{cpm} / \mu\text{mol}} \frac{(200)(20)}{(15 \lambda)(15)}$$

$\mu\text{mol turnover} =$
200 $\mu\text{l Rxn}$

$$\frac{\text{cpm} - \text{BK60}}{\text{cpm} / \mu\text{mol}} \left(\frac{200}{5} \right) \left(\frac{10}{2} \right)$$

$$\% \text{ turnover} = \frac{\mu\text{mol turnover}}{\mu\text{mol turnover} + \mu\text{mol incorp}}$$

121 75821.00
122 104512.00

To Page No. _____

Read & Understood by me,

Michael Polay

Date

11/29/94

Invented by

Recorded by

Date

11-10-94

DEI + AS $(\text{NH}_4)_2\text{SO}_4$ ppt.

in to continue with purification - following the ~~06/15/98~~
the protocol as in wild type Tne - p.108.

11 - 6.8 mL $(.05)(6.8) = 2M \times$ $x = 174 \mu\text{L}$ of 2M KCl

exo - 4.8 mL 3.8 $(.05)(4.8+x) = 2M \times$ $x = 97.4 \mu\text{L}$ of 2M KCl

$(.40)(6.8+x) = 10\% \times$
 $291 \mu\text{L} = x$ $x = 291 \mu\text{L}$ 10% DEI

$(.40)(3.9+x) = 10\% \times$
 $143 \mu\text{L} = x$ $x = 143 \mu\text{L}$ 10% DEI

Make each a Anal 50mM KCl slowly add 20% a 10%
PEI sol'n to a Anal [3] of .4%. vortex - let shake
30 minutes @ 4°C. spin in 2mL eppendorf in micro-
centrifuge 20 minutes @ 4°C - Save Supernatant.

60% $(\text{NH}_4)_2\text{SO}_4$ fractionation

TX1 $\frac{36g \text{ solid}}{100 \text{ mL}} = \frac{x}{4.8 \text{ mL}}$ 2.45g

3'-5' x10- $\frac{36g}{100 \text{ mL}} = \frac{x}{3.5 \text{ mL}}$ 1.26g

vortex - let shake 30min @ 4°C
spin in 55-34 - 20,000 x g -
Decant + Save Supernatant - Pellet's

To Page No. _____

sed & Understood by me,

May 20/95

Date

6/20/95

Invented by

E. H. H. H.

Recorded by

Date

06/16/95

From Page No. _____

Bump Heparin with .5M NaOH - Wash w/ H₂O extensive
 Equilibrate w/ Buffer A -
 Buffer A - Heparin - Buffer B - Heparin

25mM Tris pH 7.4

10% glycerol

5mM Bme

.1mM PMSF

.1mM EDTA

10mM KCl

conductivity - 1.2mS

A.S.

TY-1 - Dissolve Pellet in 10mL of Buffer A

4.5mS - conduct

Add 30mL additional of Buffer A

2.1mS - conduct

Load 9 35mL on 2mL TBSO Heparin @ .75mL/min
 collect flow through material - wash to base line -

Gradient Program - 0 - 100% B @ .5mL/min - 20mL linear
 wash 100% B - 10mL - @ .5mL/min
 collect 500 μ fractions -

To Page

Witnessed & Understood by me,

Date

Inv. nted by

Date

Recorded by

Mam Jones

6/20/25

6/15/25

6/15/95

ag N _____
Mtx Rxn

tock

For 20 mL

SMTAPS

1 mL

50 mM MgCl₂800 μ L

2M KCl

500 μ L

1 M DTT

200 μ L

10 mM dNTPs

400 μ L

ct. Salmon testes

5 mL

12.1

- 1.1 mL dCTP
vial

20 mLs

Aliquant 500 μ L / tube store in -20°C freezer - yellow tubes -

To Page No. _____

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Date

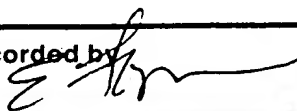
Invented by

Date

Nay Long

6/20/95

Recorded by



6/16/95

Project No. _____

Book No. _____

TITLE Heparin - FY-1

142

From Page No. _____

06/15

SAM

CPM1

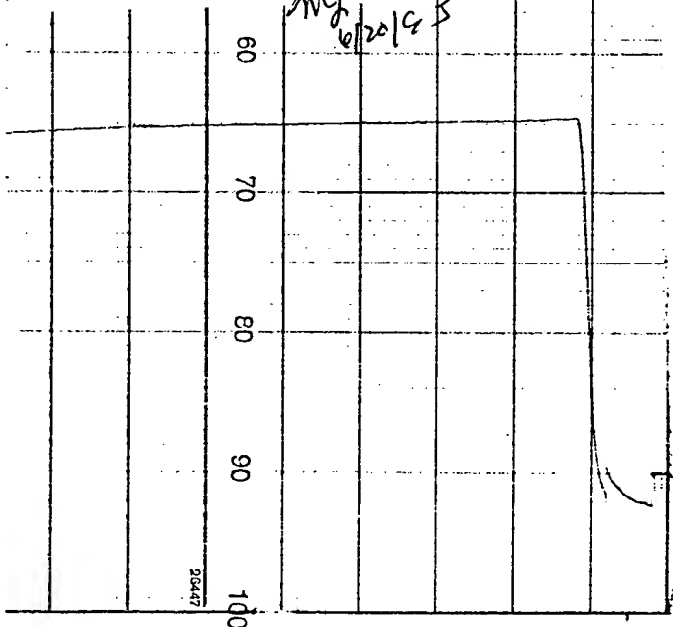
EX

FY-1

| | |
|----|-------------|
| 1 | 115552.0052 |
| 2 | 53328.0054 |
| 3 | 9146.0056 |
| 4 | 4556.0058 |
| 5 | 1260.0059 |
| 6 | 3744.0060 |
| 7 | 1028.0061 |
| 8 | 574.0062 |
| 9 | 536.0063 |
| 10 | 346.0064 |
| 11 | 730.0065 |
| 12 | 438.0066 |
| 13 | 348.0067 |
| 14 | 21268.0068 |
| 15 | 668.0069 |
| 16 | 372.0070 |
| 17 | 866.0071 |
| 18 | 74836.0072 |
| 19 | 146.00 |

Pool 47-55 dialyze O/N in Queso Buffer A

my 6/20/95



Pharmacia LKB Biotechnology

2.4 µl Rxn
 1 µl pack
 Sample -
 incubate @
 in 8' - qu
 w/ 10 µl of S
 EDTA - SP
 20 µl on t
 wash
 5' 1x 10' TCI
 3' 3x 5' T.
 2x S to
 dry + cou
 econoflow

Pool - 49
 dialyze O/
 in again
 Queso Buf
 See p. 144

11111
 7

With ss d & Understood by me,

Date

Invented by S. Kym

Date

To Page N

Man Jongo

4/20/95

R corded by

06/16/95

Hepairin 3-5 cno mutant

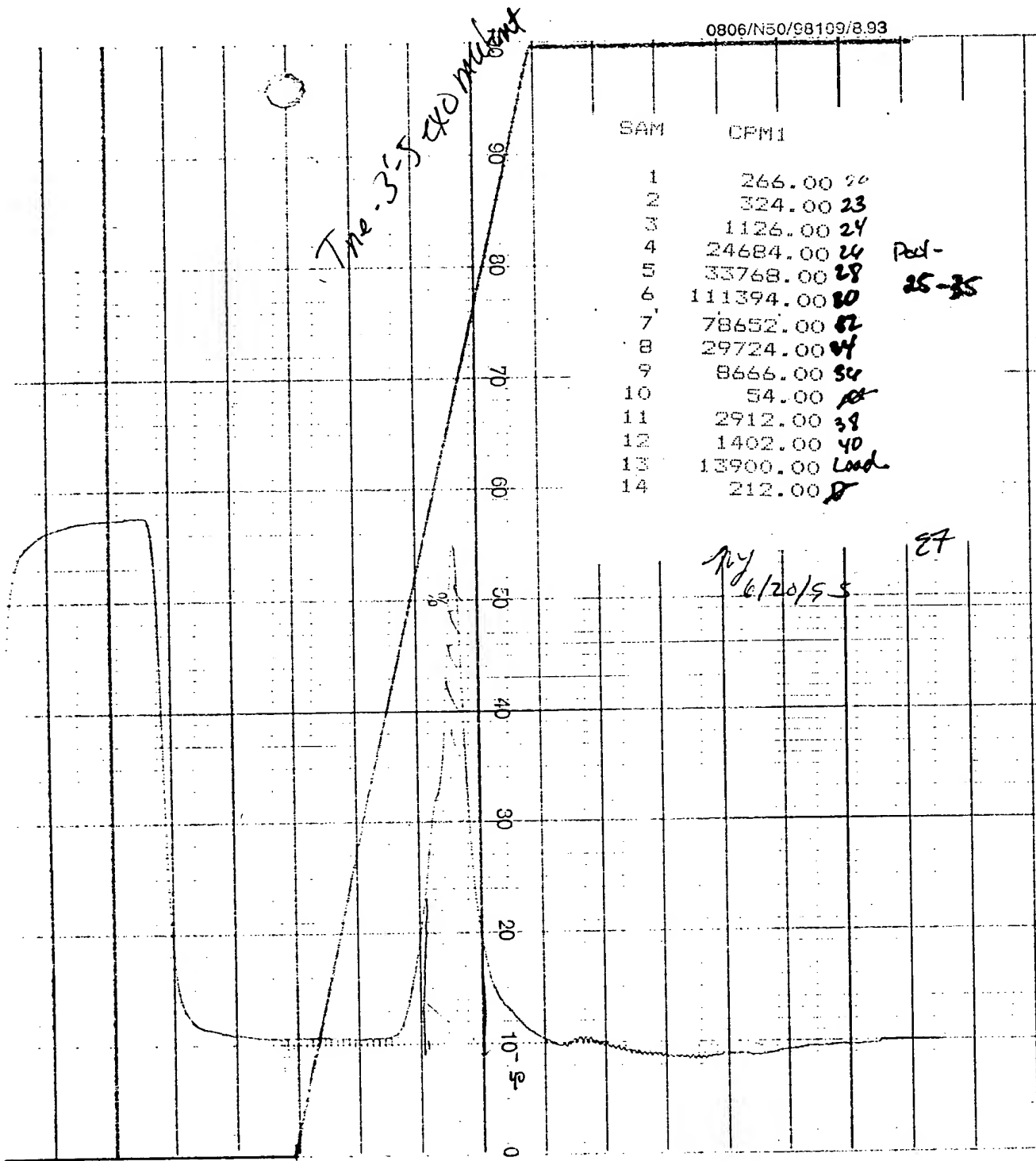
Project No. _____

Book No. _____

143

ag No. _____

06/15



24 μ l mix
1 μ l fraction
Sample -
incubate @
74° 8 min -
quench w/
10 μ l g. SM
EDTA -
Spot 20 μ l
on GF/C
Wash -
1x 10% TCA
1x PC
3x 5% TCA
2x EtOH
dry +
count -
Pool - 25-35
dialyze 4 hrs
in QLSO
Buffer A \rightarrow
See p. 148

6/20/95

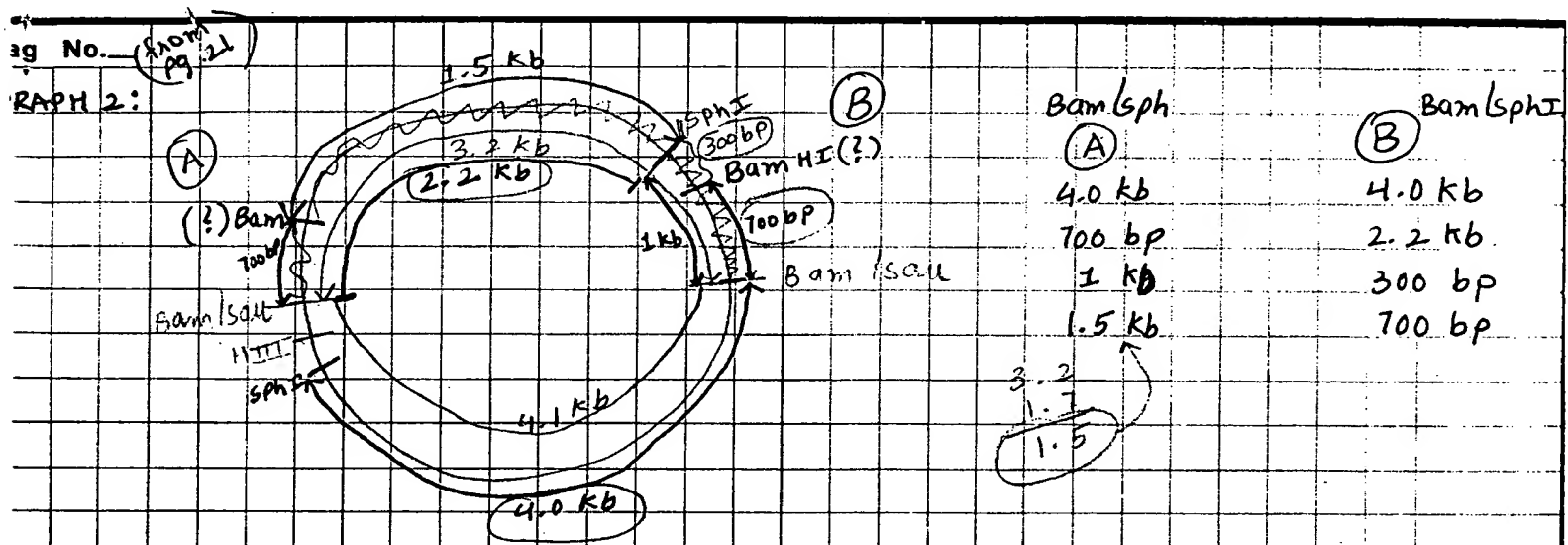
6/15/95

Technology Code No. 18-1001-44

any 6/20/95
sed & Understood by me,
May 16/95

| | | |
|-----------------|-----------------------------|-----------------|
| Date
6/20/95 | Invented by
Elizabeth Ly | Date
6/16/95 |
| Recorded by | | |

To Page No. _____

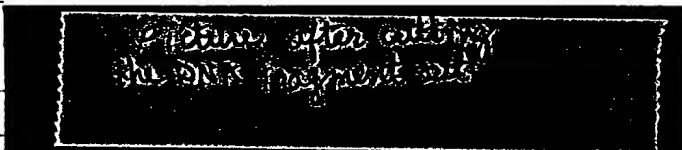
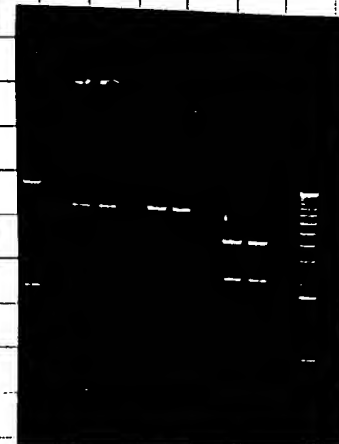


Cloning mp 18 w/ T.nea/psport & mp 19 w/ T.nea/psport

3/2/95

3/2/95

Thurs.



DID GENE CLEAN.

To Page No. _____

sd & Understood by me,

Date

Invented by

Date

Recorded by

4/12/95

4/14/95

[Signature]

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From Page No. _____

GENE CLEAN

- Mixed mp 18 with T_{ne}a pSPORT cut w/ Sst I sph } 1 tube
- " mp 19 with " " " " " } 2 tube

- added 700.0 μ l NaI to each 2 tubes. Vortexed
- put the tubes in 55°C heat block to melt agarose
- after agarose melted, added 5.0 μ l glass milk to both tubes
- incubated both tubes on ice for 5.0 min.
- c.f.g. both tubes (quick spin)
- discarded supernate & washed pellet 3 x with New Wash 6
- added 14.0 μ l dH₂O to each tube
- quick spinned, discarded pellet & saved supernate.

Set-up Ligation

- (mp 18) (mp 19) DNA - 14.0 μ l
- (ligase) 5x buffer - 4.0 μ l
- ligation - 2.0 μ l.
- TV - 20.0 μ l.

- incubated both

Transformation Cells

- (1) 100.0 μ l Competent
- 3.0 μ l DNA (from ligation)

- (2) incubated on ice for 30 min.

- (3) heat shocked @ 42°C H₂O bath for 35 sec.

- melted 0.7% 2x YT top agar, added 4.0 μ l to 6 different glass tubes & put the tubes @ 55°C heat block

Did not work

T Pag No

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Dat

4/12/95

Inv nt d by

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Dat

4/12/95

Project No. _____

Book No. _____

TITLE _____

124 12/6/94

From Page No. _____

Purpose: To try the new scheme! in 8 days!! 10 hrs
18 hands!!

I Vector prep: pvc 19 - 100 μ y *
0.45 μ y/l peeled from several tubes

- extracted with Aak II ① - 1 hr = 8 hr
ECORI & Bam HI ② - 1 hr = 3
Afl III ③ - 2 hr = 2
successively at 37°

LT1 pvc 19 100 μ y 225 μ l
NEB Buffer 4 (10x) 40
NEB Aak II (240/1) 10
TE 125

5 μ y added
1.25 μ y loaded

400 → 20 μ l saved after 1 hr at:

LT1 Aak II 403 EORI } 20
74121 Bam HI } 20
(0.6/1)

440

4.5 μ y
1.075 μ y

LT1 Afl 3 & D1 (7403) } 30
BMB 101 }

5' A' C P u r y G T-3'
3' T G P y p u c A-5'

470

90 μ y

2 hr at 37°

Run a, b, c on minigel along with uncut pvc and
carrier / Hind III marker.

5 μ l of a, b & c, uncut pvc 0.5 \times = 0.225 μ y

Final (C) stored at 4° overnight.

To Page N

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12/10/94

Invented by

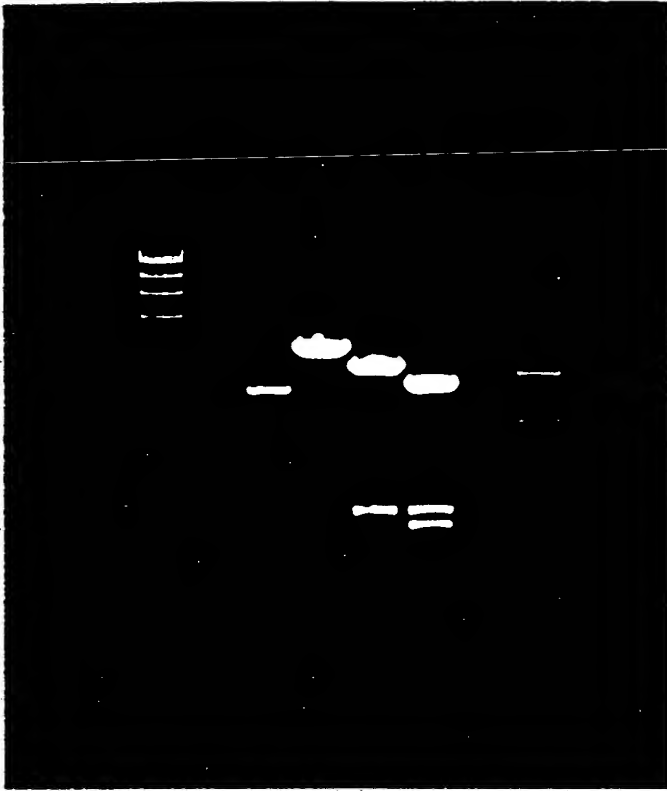
Recorded by

K. Sivarman

Date

12/11/94

Page No. _____



unclut pvc Aak II EcoRI BamHI Apl 3

pvc = 2686 bp

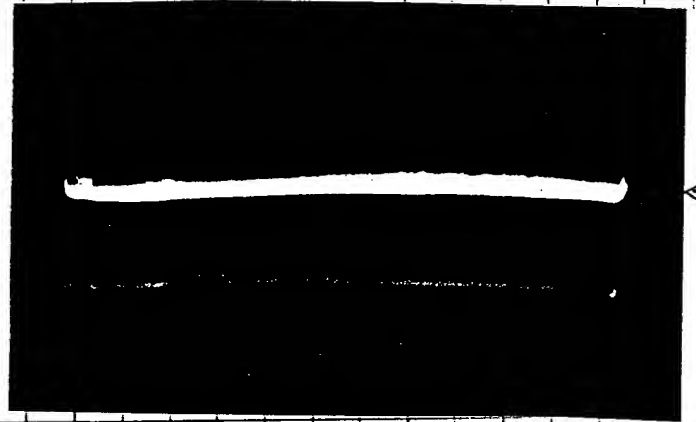
Aak II @ 26/17 + Apl II @ 806 = Vector = 1811 bp 0.6742 / 1
cut = 875 bp 0.325

DEAE paper containing the vector band, washed in { 1M NaCl
for 10 mM Tris 8.
5 mM EDTA

ethanol ppt (Tol) "in" = 100 ng / 1

- Digestion seems to be complete

- All enzymes are potent!



- loaded in 40 µg of cut plasmid 19
and purified the vector band
- is 26.97 µg.

To Page No. _____

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Date

12/19/94

Invented by

Recorded by

Dr. Srinivasan

Date

12/7/94

Project No. _____
B ok No. _____

TITLE puc / F&R non du transform

126 12/7/94

From Page N _____

Prep: To transform puc / F&R non du primers.

- amplified, ethanol pptd, cut with Dat II again
linearized with Dat II & ligated
- 2⁵ µl of ligation mix transformed with DH5α max if cells.
- Plated 25, 50, 100 µl from each reaction (from 5

Treated 4 different Rx: all prepared by Judy and

| | | | | | |
|---|----------------------|-----|-----|-----|----------------------|
| ① | Taq, 1.5 mM Mg | 25 | 17 | - | |
| | (395) | 50 | 9 | - | |
| | | 100 | 17 | - | all blue
no white |
| ② | Taq, 2 mM Mg | 25 | 2 | - | |
| | (386) | 50 | 1 | - | ? |
| | | 100 | 3 | + | |
| ③ | Taq + D.V. 1.5 mM Mg | 25 | 10 | 6 | 60% |
| | (403) | 50 | 25 | 17 | 68% |
| | | 100 | 24 | 13 | 54% |
| ④ | Taq + D.V. 2 mM Mg | 25 | 136 | 40 | 29% |
| | (404) | 50 | 223 | 82 | 37% |
| | | 100 | 405 | 141 | 35% |

Result: Taq + D.V. unusually high error - mutation

Taq alone * of colonies too low, but whatever is there not much white though

nothing makes sense

To Page N _____

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Invented by

Date

Recorded by

A. Johnson

12/8/94

Project No. _____ Book No. _____ TITLE Repeat unit assay QC for v129
lot # EKBT1 done on P 61 expt

From Page No. _____
amplitude lot # 9957 for control
lot EKBT1 is ~401 u/ml based on P.61

1. starting dilutions of EKBT1:

| | |
|-------------------------------------|-------------------------------------|
| <u>1:80 (estimate cf=5%)</u> | <u>1:160 (estimate cf=2.5)</u> |
| lot EKBT1 5 μ l | 5 μ l |
| Tag storage 395 μ l | 795 μ l |
| buffer | |
| actual is 4.03%
Vf = 400 μ l | actual is 2.01%
Vf = 800 μ l |

2. 1/600 dilutions

| serial dilution # | 1-6 | 7-12 | 13-18 | 19-24 | 25-30 | 31-36 | 37-42 | 43-48 | 49-54 |
|-------------------|---------------------------|------|-------|-------|-------|-------|-------|-------|-------|
| | I | II | III | IV | V | VI | A-1 | A-2 | A-3 |
| 1:80 dil | 3.1 | 3 | 3 | | | | | | |
| 1:160 dil | | | | 3 | 3 | 3 | | | |
| Amplitude 5% | | | | | | | 3 | 3 | 3 |
| lot # | | | | | | | | | |
| dilution buffer | 1797 μ l | | | | | | | | |
| | Vf = 2000
1800 μ l | | | | | | | | |

Vortex 5s
use from 20 to 40 min

dilute I - A-3 as shown for I below:

3. Serial dilutions

| serial dilution # | dilution buffer | |
|-------------------|-----------------|---------------|
| 1 | 100 λ | 300 λ |
| 2 | 100 λ | 300 λ |
| 3 | 100 λ | 300 λ |
| 4 | 100 λ | 300 λ |
| 5 | 100 λ | 300 λ |
| 6 | 1 ml of I | 300 λ |

dilute I - III and assay
 then dilute IV - VI and assay
 then dilute A-1 - A-3 and assay

SA I-III = 45 μ l assay mix + 5 μ l dil buffer, do same for IV-VI
 spot 4x 5 μ l on 6 FC in 4 quadrants
 Blank is 45 μ l assay mix + 5 μ l dil buffer - spot on GFC along with other

Page No. _____

55-57 = Blank for I-III, IV-VI and A1-A3 respectively

58-61 = SA for I-III

62-65 = SA for IV-VI

Result:

using amphotas lot #9957 here gives a
unit value of ~~320 u/pl~~ 323.4 u/pl
compared to 401 u/pl (found on P, 61, 10-1-94)

To Page No. _____

s d & Und rstood by m ,

Sandra Pokay

Date

11/6/95

Invented by

Recorded by

Date

10-15-94

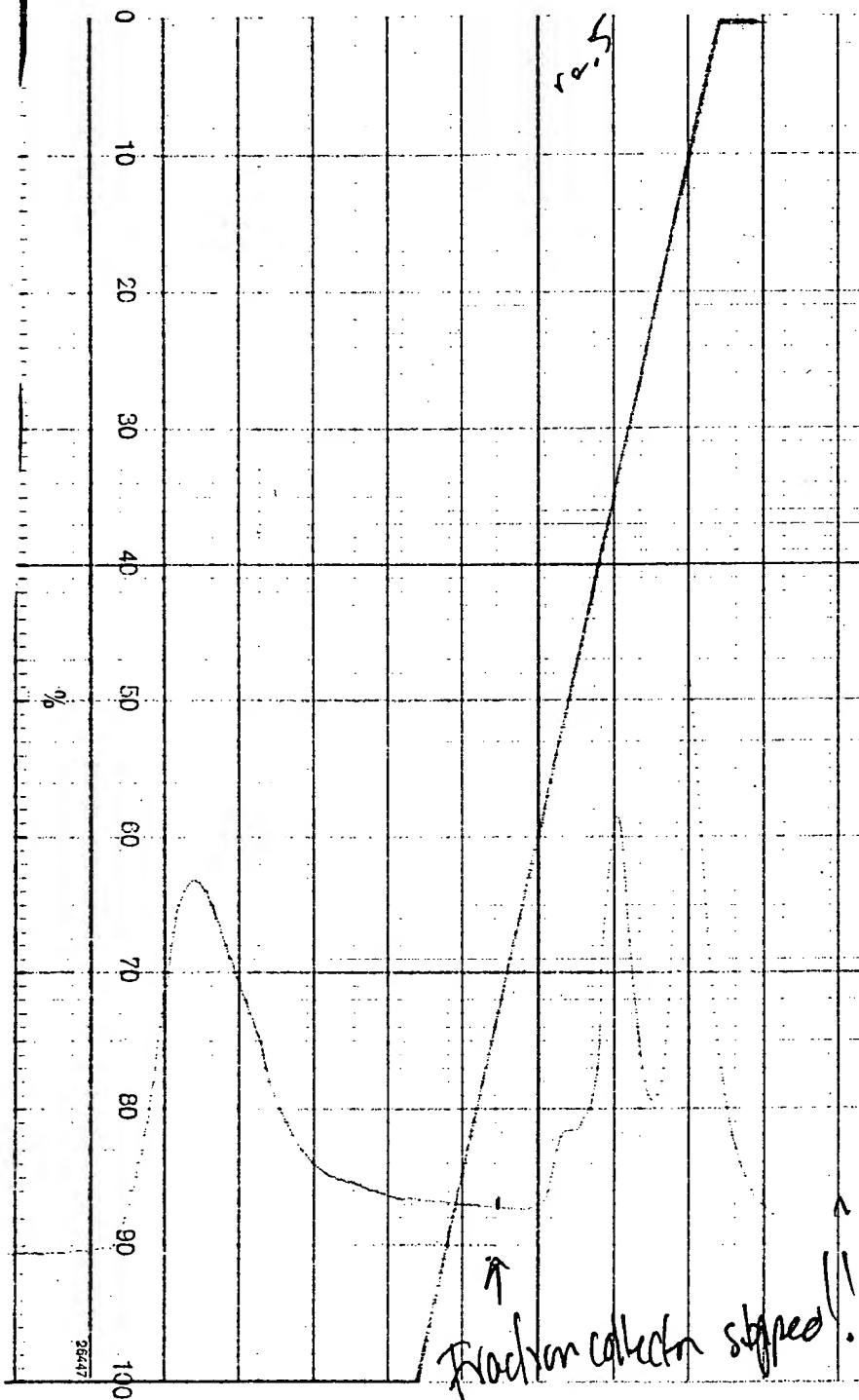
Project No. _____
Book No. _____

TITLE FY-1 Q6SDM-2ml

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06/11



Q Buffer A -

25mM Phos - pH 7.2
.1mM EDTA
10mM KCl
5mM Bme
10% glycerol

Q Buffer B

25mM Phos - pH 7.2
.1mM EDTA
800mM KCl
5mM Bme
10% glycerol

6/20/95

Pharmacia LKB Biotechnology

Code No. 18-100

To Page 1

Witnessed & Understood by me,

Date

Invented by

Date

May Longo

6/20/95

Recorded by

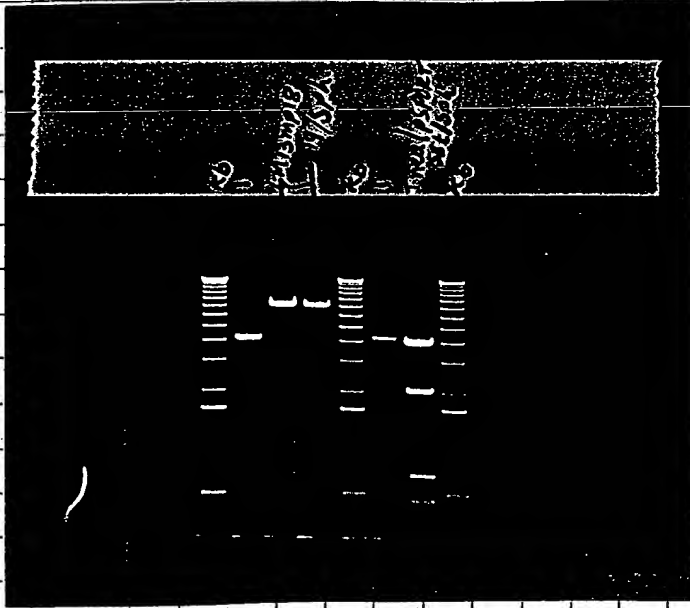
S. Hyman

06/16/95

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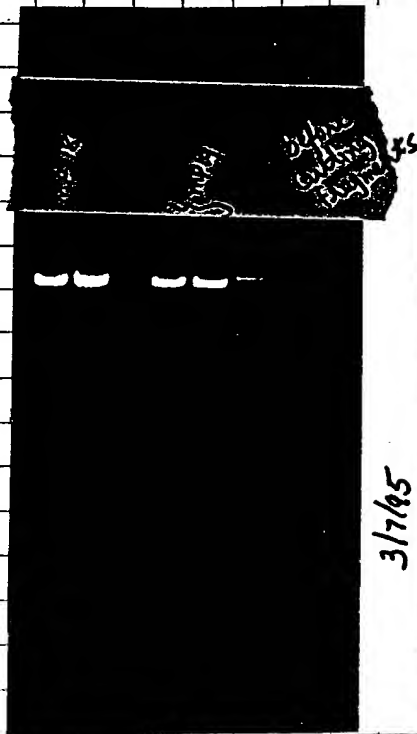
Repeat

3/7/95 TUE

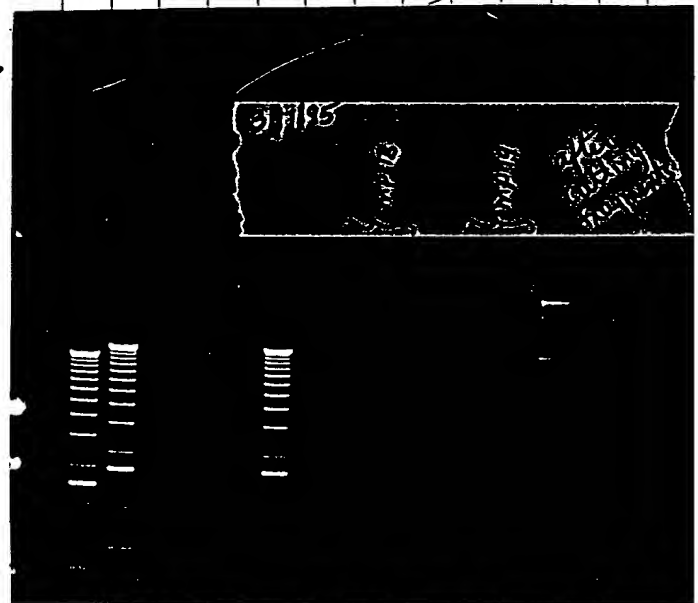


3/7/95

After taking picture on looking @ the gel, ~~M13~~ * M13 mp 18 and M13 mp 19 is @ the 7.2 kb ~~cut~~ which was cut with Sph I. ~~we decided to cut~~ We planned on cutting mp 18 and mp 19 with Sst I. The gel ^{picture} below shows mp 18 & mp 19 before & after cutting the DNA fragments. After cutting the fragment performed Gene CLEAN.



3/7/95



To Page No. _____

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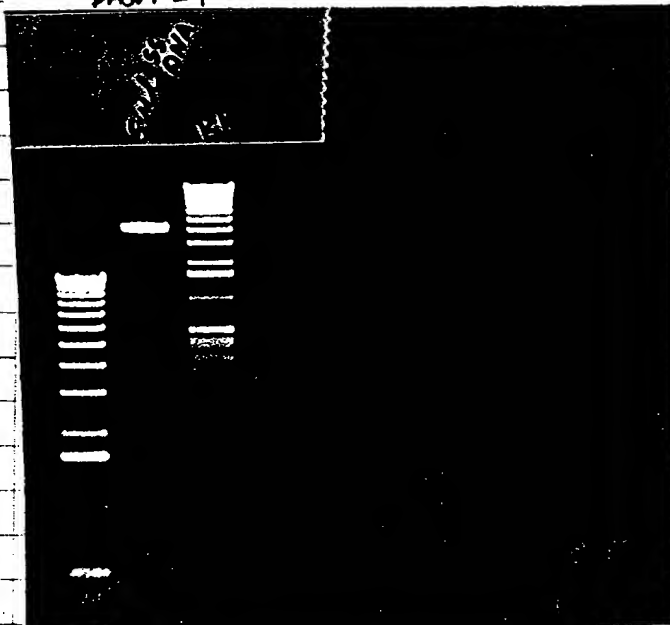
From Page No. _____

labelled 2 tubes, 1 w/ mp 18, & 2nd w/ mp 19

1. to the DNA w/ agarose gel, added 100.0 μ l NaI
2. put the tubes @ 52°C heat block to melt agarose, vortexed constant
3. added 5.0 μ l glass milk to both tubes - mixed
4. incubated on ice for 5 min.
5. (fg. (quick spin) @ room temp.
6. discarded supernate, added 500.0 μ l New wash buffer
7. discarded supernate, washed pellet 3x with New wash buffer
8. after washing 3x, added 14.0 μ l dH₂O to the pellet (discarded & (mixed)
9. incubated @ 52°C for 5 min.
10. discarded pellet & saved supernate for ligation.
(could this on 3/8/95 wed.)

Purification of m13 ssDNA (T. res 2 kb [SphI] / mp 19) from pg. 1

T. res (mp 19) ssDNA (SphI)
DH5 α 50



amp
4/12/95
R

To Page 1

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Date

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4/12/95

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cell growth & infection

- Grew an *E. coli* F' strain to an OD of 0.2-0.4 in 2x YT
- Inoculated 1-2 ml of the cells w/ the phage (added 10.0 μ l from a liquid phage stock & added to cells)
- Incubated the phage infected cells @ 37°C for 5-7 hours.
- The supernate can now be processed for isolation of ssDNA & the cells can be processed for the isolation of Replication Form (RF) dsDNA.

Purification of ml3 ssDNA

- transferred 1.0 ml culture of infected cell to 4 different eppendorf tubes
- cfg 4 tubes for 2 min.
- transferred supernate to the new tubes & saved pellet from 1 tube (out of 4 tubes) for isolation of RF DNA
- Spinned the supernate again & transferred the supernate to the new tubes (done to remove any residual cells remained behind)
- passed the supernate through a 0.45 μ filter as to remaining cells (done when performing site-directed mutagenesis)
- added 200.0 μ l of 20% PEG + 1.5 M NaCl. Vortexed
- Incubated tubes for 15 min @ room temperature (or overnight @ 4°C)
- cfg for 10 min in a 4cfg. @ room temp.
- discarded supernate & briefly spinned the tubes to remove the residual soln from the side of the tube (removed as much ^{supernate} as possible)
- added 200.0 μ l TE. Vortexed
- cfg for 2 min. to remove any residual cell debris.
- Transferred supernate to the new tube. (added 5.0 μ l RNase I to remove any residual nucleic acid from the prep. Beryonase will remove both RNA & DNA very efficiently.)
- added equal volume of phenol/chloroform/isoamyl alcohol. mixed well.
- cfg for 5.0 min.
- transferred the upper layer to a new tube (BE CAREFUL NOT TO DISTURB WHITE INTERFACE OR REMOVE ANY PHENOL)
- added 20.0 μ l NaAc & 600.0 μ l EtOH
- Incubated @ -70°C for 5-15 min. (we left @ -70°C overnight)

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Date

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Date

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4/12/95

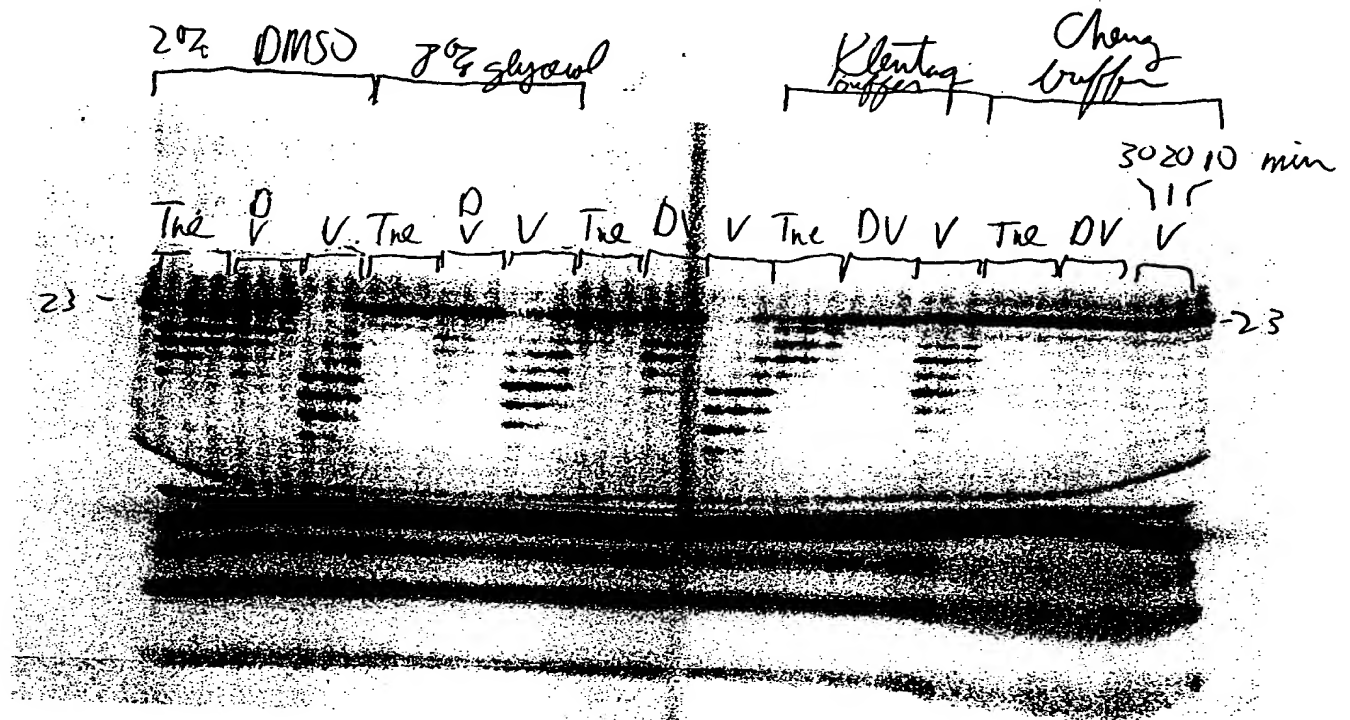
Recorded by

4/12/95

82

Project No. _____
Book No. _____ TITLE _____

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Result.

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Witnessed & Understood by me,
Deena Polay

Date
11/29/94

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Recorded by

Date
11-5-94

Project No. _____

Book No. _____

TITLE _____

82

From Page No. _____

| 20% DMSO | | 80% glycerol | Klentag buffer | | Cheng buffer |
|---|----------------------|-------------------|-------------------|-------------------|-------------------|
| Tissue pH 8.7 | Trio-Hell pH | pH | pH | pH | pH |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| K ⁺ el | K ⁺ el | K ⁺ el | K ⁺ el | K ⁺ el | K ⁺ el |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| (NH ₄) ₂ SO ₄ | Mg(OAc) ₂ | MgSO ₄ | DMSO | Trehalose | Trehalose |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| Trehalose 20% w/v | glycerol | Trehalose | Trehalose | Trehalose | Trehalose |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| Vant | Deep vent | Trehalose | Trehalose | Trehalose | Trehalose |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

Re

(+ for OAc)

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Deena Polay

Date

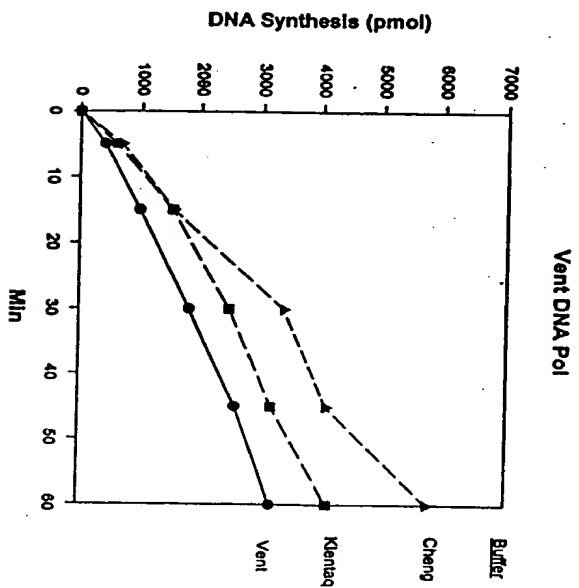
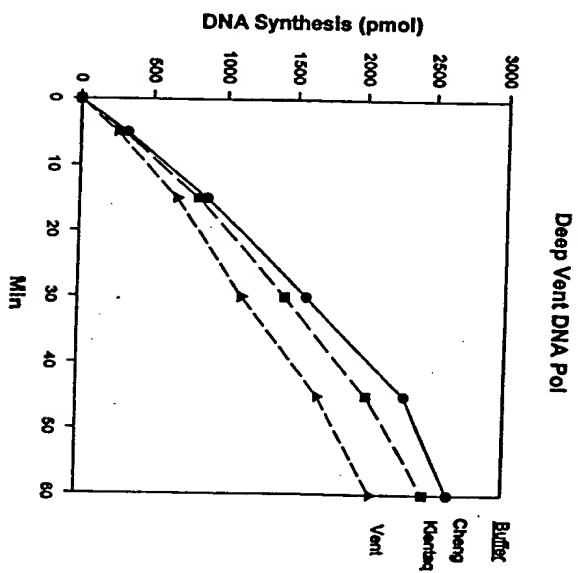
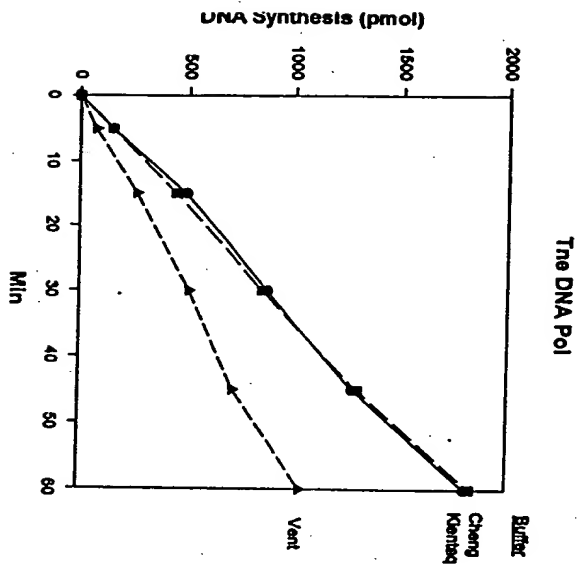
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Date

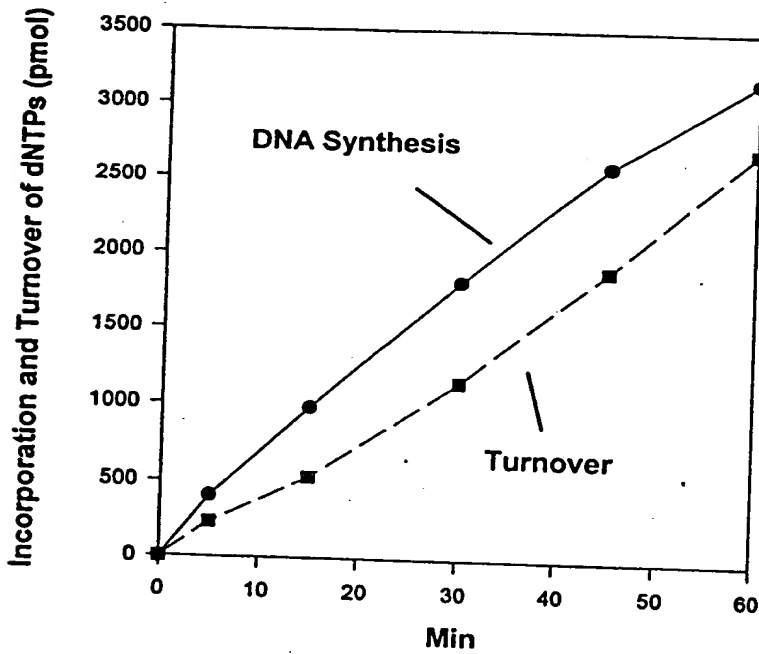
11/5-94



In each case, DNA synthesis is lower in
 Primer degradation was highest in Vent

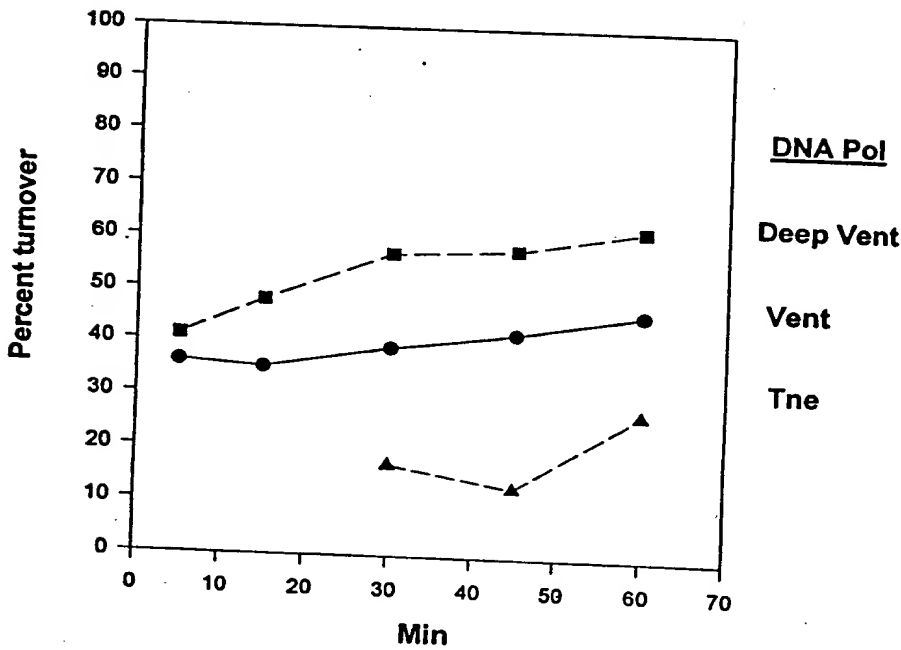
got Turnover
 by DNA synthesis
 1. label

Vent DNA Pol in Vent Buffer



DNA synthesis
and turnover
to dNMP

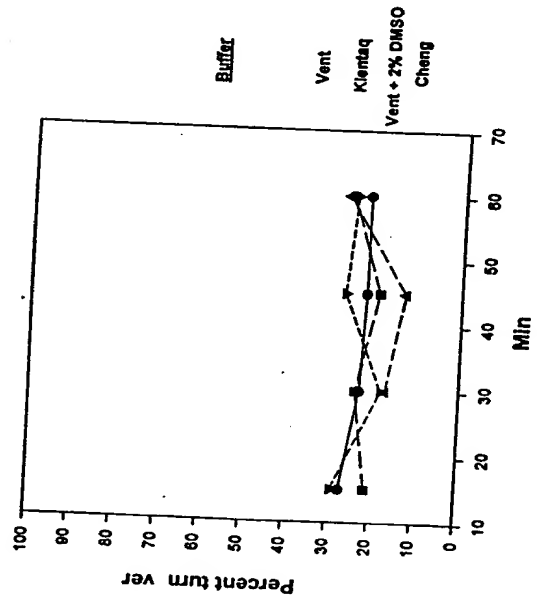
Activity in Vent Buffer



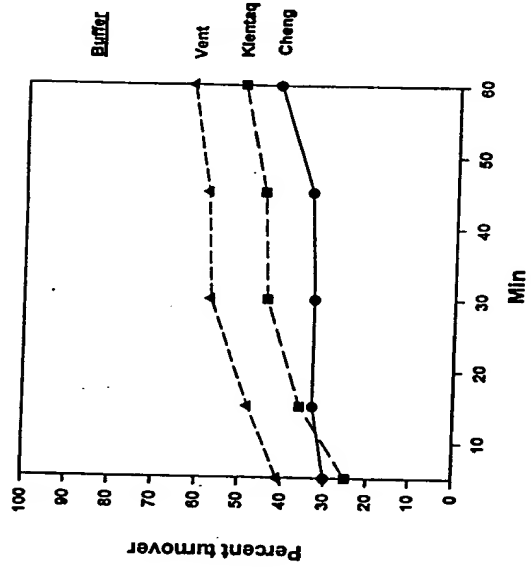
Percent turnover =
 $\frac{\text{turnover}}{\text{incorporation} + \text{turnover}}$

Deep Vent has
higher turnover
than Vent as
expected. Tne
is ~2x lower
than Vent and
Deep Vent

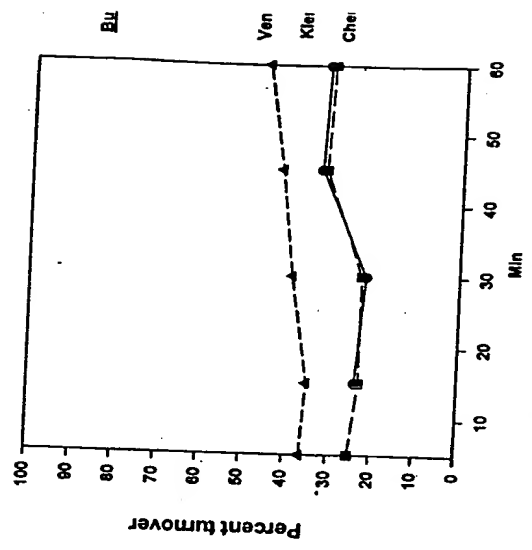
The DNA Polymerase



Deep Vent DNA Polymerase



Vent DNA Polymerase



effect of buffer on turnover is not large compared to effect on primer degradation

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a a a a a a a a a a

Date 11/29/94

Invented by

Recorded by

Date

11-5-94

Turnover for Vent, deep Vent
(follow p. 61, 7)

From Page No. _____

| | | | |
|----------------------------|--------------------|--------------------|-------|
| H ₂ O | (A) | (B) | (C) |
| 5x Cheung buffer | 399 489 | 489 467 | 489 4 |
| 10x KlenTaq | 133 | 66.7 | |
| 10x Vent buffer | | | |
| Tag storage buffer | 6.7 λ | | 66.7 |
| 3.7 mg/ml activated DNA | 90 | | |
| JATG-T-TP 10mM each | 3.33 | | |
| 32P dATP 10mCi/ml | 1.2 λ | | |
| Mg(OAc) ₂ 50 mM | 16 μ l | | |
| MgSO ₄ 100 mM | | 8 μ l | |
| DM50 100 μ l | | | |

| | | | | | | | | |
|------------------------|---------|-----|-----|-------|-----|-----|-----------------|-----|
| | 0.65 ml | | | 0.633 | | | 2.633.65 use 1. | |
| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) |
| Tag storage buffer | 195 | 195 | 195 | 190 | 190 | 190 | 190 | 190 |
| Vent 0.08 μ l | 4 | | | 4 | 4 | - | 4 | |
| Deep Vent 0.08 μ l | | 4 | | | 4 | | | 4 |
| Taq 0.07 μ l | | | 4 | | | 4 | | |
| H ₂ O | | | | | | | | |

prime to 70°C, start by addition of pol 5 6

remove 15 μ l to 5 μ l 0.2 M EDTA \rightarrow spot 15 μ l on 6
and remove 5 μ l to 5 μ l Kill solution (20 μ mol/ml dATP
100 mM EDTA) at 90°C

0 5 15 30 45 60 min
spot 2 μ l on PEI resolve in 1M LiCl

* dilutions of pols
same as P81

Results : see graph on P81

Witnessed & Understood by me,

Deena a Bolour

Date

11/29/94

Invented by

R cord d by

Dat

11-9-94

To Page 1

19 N

(1)

14.4

✓

✓

✓

66.7 20

✓

→ 27

✓

1. $\mu\text{l} / 100 \mu\text{l PCR} \Rightarrow C_f = 0.005\%$ Tween 20/NP40

So this makes up for no TPE here - its present in Jones long PCR Rxn.

→ 1

✓

(Cp = 50 μm each)

→ 0.36

✓

(220 x 10⁶ total cpm)

✓

(1.2 mM Mg(OAc)₂)

✓

(1.2 mM MgSO₄ in Klenow buffer)4 μl

✓ Cf = (2% DMSO)

(2 mM MgSO₄ in 1X Vent buffer)

(10)

19.4

✓

(0.4 units total of each pol)

4

1

To Page N _____

Designed & Understood by me,

Zachary Pokany

Date

11/29/94

Invented by

Recorded by

Date

11-9-94

86

From

Cheng

Rentag

With

Project No.

Book No.

TITLE

Incorp

Ampl

| | | | | | | |
|----|---------|--------|---------|-----|----------|------|
| 01 | 543.00 | BK60 | (14) | 61 | 269.00 | |
| 02 | 650.00 | 110 | | 62 | 7412.00 | 779 |
| 03 | 1014.00 | 486 | 24 | 63 | 16953.00 | 8553 |
| 04 | 1485.00 | 771 | 22 | 64 | 36825.00 | 3374 |
| 05 | 2627.00 | 2148 | 34 | 65 | 44610.00 | 4087 |
| 06 | 3187.00 | 2725 | 32 | 66 | 62771.00 | 5752 |
| 07 | 525.00 | BK60 | | 67 | 241.00 | |
| 08 | 662.00 | 141 | 30 | 68 | 3518.00 | 322 |
| 09 | 948.00 | 436 | 33 | 69 | 9506.00 | 871 |
| 10 | 1271.00 | 763 | 33 | 70 | 17320.00 | 1587 |
| 11 | 1677.00 | 1188 | 34 | 71 | 25050.00 | 2296 |
| 12 | 2340.00 | 1871 | 42 | 72 | 28643.00 | 2625 |
| 13 | 624.00 | BK60 | | 73 | 324.00 | |
| 14 | 694.00 | 72 | (32) | 74 | 1974.00 | 15 |
| 15 | 796.00 | 177 | 27 | 75 | 5340.00 | 489 |
| 16 | 880.00 | 264 | 23 | 76 | 9478.00 | 869 |
| 17 | 976.00 | 363 | 22 | 77 | 13880.00 | 1272 |
| 18 | 1110.00 | 501 | 22 | 78 | 19753.00 | 1810 |
| 19 | 805.00 | BK60 | 775 Ave | 79 | 321.00 | |
| 20 | 977.00 | 192 | 25 | 80 | 8826.00 | 582 |
| 21 | 1409.00 | 467 | 23 | 81 | 23029.00 | 1533 |
| 22 | 1803.00 | 762 | 23 | 82 | 37324.00 | 2485 |
| 23 | 2832.00 | 1133 | 32 | 83 | 47661.00 | 3173 |
| 24 | 3299.00 | 1883 | 31 | 84 | 61758.00 | 4112 |
| 25 | 774.00 | BK60 | | 85 | 404.00 | |
| 26 | 918.00 | 99 | 25 | 86 | 4493.00 | 299 |
| 27 | 1406.00 | 415 | 36 | 87 | 12238.00 | 815 |
| 28 | 2277.00 | 1118 | 44 | 88 | 21497.00 | 1431 |
| 29 | 2989.00 | 1651 | 45 | 89 | 30491.00 | 2030 |
| 30 | 4085.00 | 2472 | 50 | 90 | 36800.00 | 2450 |
| 31 | 777.00 | BK60 | | 91 | 214.00 | |
| 32 | 813.00 | 21 | (12) | 92 | 2257.00 | 150 |
| 33 | 947.00 | 121 | 21 | 93 | 6671.00 | 444 |
| 34 | 1136.00 | 263 | 24 | 94 | 12685.00 | 745 |
| 35 | 1204.00 | 314 | 19 | 95 | 19429.00 | 1294 |
| 36 | 1631.00 | 633 | 26 | 96 | 27534.00 | 1855 |
| 37 | 919.00 | BK60 | 922 Ave | 97 | 239.00 | |
| 38 | 1284.00 | 231 | 36 | 98 | 7128.00 | 404 |
| 39 | 1754.00 | 530 | 33 | 99 | 17335.00 | 881 |
| 40 | 2728.00 | 1150 | 39 | 100 | 32171.00 | 1821 |
| 41 | 3910.00 | 1903 | 42 | 101 | 45795.00 | 2592 |
| 42 | 5168.00 | 2704 | 46 | 102 | 56065.00 | 3174 |
| 43 | 924.00 | BK60 | | 103 | 318.00 | |
| 44 | 1205.00 | 180 | 41 | 104 | 4474.00 | 253 |
| 45 | 1892.00 | 617 | 48 | 105 | 11839.00 | 670 |
| 46 | 3234.00 | 1472 | 57 | 106 | 19756.00 | 1119 |
| 47 | 4572.00 | 2325 | 58 | 107 | 29674.00 | 1170 |
| 48 | 6365.00 | 3467 | 62 | 108 | 36540.00 | 2069 |
| 49 | 863.00 | BK60 | | 109 | 261.00 | |
| 50 | 901.00 | 20 | (7) | 110 | 1566.00 | 74 |
| 51 | 953.00 | 103 | 17 | 111 | 4647.00 | 263 |
| 52 | 1083.00 | 103 | 13 | 112 | 8879.00 | 503 |
| 53 | 1085.00 | 386 | 27 | 113 | 12496.00 | 707 |
| 54 | 1529.00 | 984.00 | | 114 | 18327.00 | 1037 |
| 55 | 984.00 | BK60 | | 115 | 295.00 | |
| 56 | 891.00 | 92 | 29 | 116 | 1709.00 | 80 |
| 57 | 1067.00 | 104 | 18 | 117 | 4261.00 | 224 |
| 58 | 1086.00 | 264 | 25 | 118 | 8343.00 | 492 |
| 59 | 1336.00 | 347 | | 119 | 12504.00 | 708 |
| 60 | 1467.00 | | | 120 | 18443.00 | 1040 |

Invented by

Record d by

11/29/94

1/9-94

g N . _____

JAMP BK60%

1. Cherry mix = 564 ave
2. Blent mix = 785
3. Vent mix = 922

Spot
Cherry

$$75821 \text{ CPM} \left(\frac{50 \mu\text{L Rxn Vol}}{2 \lambda \text{ spotted}} \right) \left(\frac{200}{195} \right) \left(\frac{1}{2500 \mu\text{m}} \right) \left(\frac{1}{4} \right) = 194 \frac{\text{CPM}}{\mu\text{mol}}$$

Blent

$$267 \frac{\text{CPM}}{\mu\text{mol}}$$

Vent

$$314 \frac{\text{CPM}}{\mu\text{mol}}$$

pmol incorp =
(200 μL Rxn)

$$\frac{\text{CPM}}{\text{CPM}/\text{pmol}} \left(\frac{200}{15} \right) \left(\frac{20}{15} \right)$$

pmol turnover =
200 μL Rxn

$$\frac{\text{CPM} - \text{BK60}}{\text{CPM}/\text{pmol}} \left(\frac{200}{5} \right) \left(\frac{10}{2} \right)$$

To turnover = $\frac{\text{pmol turnover}}{\text{pmol turnover} + \text{pmol incorp}}$

21 75821.00
22 104512.00

To Page No. _____

& Understood by me,

Eric Polay

Date

11/29/94

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Date

11-10-94

PAGES 88-89 OF NOTEBOOK WERE BLANK

AT 9411 Carried Project No. _____
 90 out of Frederick Book No. _____

Repeat unit assay QC for v124
 lot # EKBT1 done on P 61 spec

From Page N _____

Amplitag lot # 9957 for control
 lot EKBT1 is ~ 401 u/ml based on P.61

1. starting dilutions of EKBT1:

1:80 (estimate cf=5%)

1:160 (estimate cf=2.5)

lot EKBT1 5 μ l

5 μ l

Tag storage 385 μ l
 buffer

385 μ l

actual is 4.03 u/l

actual is 2.01 u/l

Vf = 400 μ l

Vf = 800 μ l

2. 1/600 dilutions

| serial dilution # | 1-6 | 7-12 | 13-18 | 19-24 | 25-30 | 31-36 | 37-42 | 43-48 | 49-54 |
|----------------------|-----|------|-------|-------|-------|-------|-------|-------|-------|
| | I | II | III | IV | V | VI | A-1 | A-2 | A-3 |
| 1:80 dil | 31 | 3 | 3 | | | | | | |
| 1:160 dil | | | | 3 | 3 | 3 | | | |
| Amplitag 5%
lot # | | | | | | | 3 | 3 | 3 |

dilution buffer 1797 μ l
 Vf = 2000
 1800 μ l

Vortex 5A
 use from 1h
 20 and 40 ml

dilute I - A-3 as shown for I below:

3. Serial dilutions

| serial dilutions # | dilution buffer | |
|--------------------|-----------------|---------------|
| 1 | 100 λ | 300 λ |
| 2 | 100 λ | 300 λ |
| 3 | 100 λ | 300 λ |
| 4 | 100 λ | 300 λ |
| 5 | 100 λ | 300 λ |
| 6 | 1 ml of I | 300 λ |

dilute I - III and assay
 then dilute IV - VI and assay
 then dilute A-1 - A-3 and assay

SA I-III = 45 μ l assay mix + 5 μ l dil buffer, do same for IV-VI
 spot 4x 5 μ l on 6 Fc in one aqueous

Blank is 45 μ l assay mix + 5 μ l dil buffer - spot on GTC along with other

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Deereena Pokar

Date

1/6/95

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Date

11-15-94

To Page N

ag N _____

55-57 = Blank for I-III, IV-VI and A1-A3 respectively

58-61 = SA for I-III

62-65 = SA for IV-VI

Result:

using amphotag lot #9957 here gives a
 unit value of ~~323.4~~ 323.4 u/ml
 compared to 401 u/ml (found on P.61, 10-1-94)

To Page No. _____

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Sandra Pokany

1/6/95

11-15-94

Project No. _____

Book No. _____

TITLE

New definition of EKBT1 to
10⁶/μl for Larry Morley

From Page No. _____

* will use old unit value of 401 u/μl no can use
old definition of Jerry used in October
(see P 91 where final unit determination for EKBT1
is 323.4 units/μl)

calibrated P20 (P20
its capacity, 10 μg for
for P1000 (P1000) use
37 μl which gives
391 μg

Tag storage buffer 391 μl

Tag lot # EKBT1
("401" u/μl)

10 μl

* see above

$$VP = 401 \mu\text{l} \quad \left(10 \frac{\text{units}}{\mu\text{l}}\right)$$

1. Bring Tag storage buffer to room Temp.
2. Bring small aliquot aliquot of EKBT1 (main stock)
to room Temp.
3. deliver 10 μl Tag into 391 μl storage buffer, rinse
~10 times (i.e. triturate)
4. mix with P1000 to get in all storage buffer
5. vortex 5 sec
6. mix end over end in cold room 2 hr

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Deeana Polamp

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1/6/95

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11-30-94

Tag No. _____

Equilibrate 2mL Q650 m w/ Q buffer A - 6/17
dilute load of 3

Load @ .5 mL/min - ~~same~~ Sensitivity - .05

Wash to base w/ Buffer A - collect F.T. - @ 1 mL/min

Program - ① 5mL wash w/ Q Buffer A @ .5 mL/min
② 20mL linear gradient 0 → 100% Q Buffer B
@ .5 mL/min
③ 45mL wash w/ Q Buffer B @ .5 mL/min
collect 500 μ L fractions -

Assay Activity

10 μ L of premix aliquotted to pre-labeled ependarfs -
incubate @ 74°C for 5 min quench w/
10 μ L of .5M EDTA - spot 3 20 μ L on 6 F1 C
filters - TCA wash

1x 10% TCA + 1% PI @ 5'

3x 5% TCA + @ 5'

2x EtOH

dry + count in LSF - Econofluor

Pool 24-35 dialyze o/N (over weekend) - against
tag storage buffers (No detergents) -

1 - Remove ~ 1.8mL from dialysis - store in 2mL eppendorf
HOT PINK - -20°C

To Page No. _____

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Way Fargo

6/20/95

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S. H. M.

6/19/95

Q650 - TBSO kit - 2mL column

Project No. _____

Box No. _____

147

Tag N. _____

06/16

* FY-1

Wash + column w/ .5N NaOH -

Wash extensively w/ H₂O

equilibrate w/ Q650-Buffer A - p. 146 -

Load ~ 3.5mL of Heparin pool of FY-1 @ .5mL/min

Wash with QBuffer A until baseline is reached -

Gradient - 20mL linear gradient 0-100% Qbuffer B
@ .5mL/min collect .5mL fractions

Wash w/ 10mL of 100% Qbuffer B - collect
.5mL fraction fractions -

Fraction collector - started then stopped after
fraction 10/11 - Did not realize until
gradient was finished - lost entire elution
~~elution~~ to waste! Could have tried to
save however I believe I washed the port
with .2N NaOH + in the same waste container.

Fraction collector stopped b/c outside of rack was "dirty"
and was slipping - Must be sure outside plastic is
clean!

Can to proceed with 3'-5' exo mutant - Flush column
with 3M KCl - Wash w/ H₂O equilibrate w/
Q650 Buffer A p. 146.

To Page No. _____

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May Longo

9/20/95

Recorded by

06/14/95

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Book No. _____

TITLE Q650M - 3'5' x 0 minus - T

From Page No. _____

0-800mm KCl in ① pH 7.2

0806.N50/93109/8.93

| SAM | CFM1 |
|-----|------------|
| 1 | 20 188.00 |
| 2 | 22 138.00 |
| 3 | 24 180.00 |
| 4 | 26 874.00 |
| 5 | 28 830.00 |
| 6 | 30 748.00 |
| 7 | 32 1174.00 |
| 8 | 34 912.00 |
| 9 | 36 556.00 |
| 10 | 38 590.00 |
| 11 | 40 340.00 |
| 12 | 42 326.00 |
| 13 | 44 370.00 |
| 14 | 46 266.00 |
| 15 | 48 298.00 |
| 16 | 50 186.00 |
| 17 | 9928.00 |
| 18 | 198.00 |

Fracta

Pool

Pool 26-35

6/20/95

6/20/95

Pool 26-3

dialyze of

in JFAQ

storage

6/16/95

6/16/95

6/16/95

6/16/95

iotechnology

Code No. 18-1001-44

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Date

To Page N

Man Jones

6/20/95

6/20/95

6/20/95

6/20/95

6/20/95

6/19/95

6/19/95

Page No. _____

purpose: continuation of pg 124 - 125

amplified linearized puc / xmr 1 using 2 different new sets of primers

36

37

38

39

tried with Tag and Tag + DV

tested Mg 1.5, 2.0, 2.5, 3.0 mM cycling: 94° 30" 1
(94° 30" 30" 68° 1' 30")

200 μM dNTP
.4 μM primer
1 U of enzyme - Tag
25 pg template
product = 1.275 kb

prepared 10x of each: Tag / # 3 Tag + DV / # 3
" / # 2 " / # 2

| | | | | | | |
|------------------|-----|----------------|------|----|------|------|
| H ₂ O | 338 | 330 μl | | | | |
| ox buffer | 50 | | | | | |
| dNTP | 10 | | 1.5 | 2 | 2.5 | 3 mM |
| Mg | - | | 7.5 | 10 | 12.5 | 15 |
| primer 1 | 20 | | 42.5 | 40 | 37.5 | 35 |
| 2 | 20 | | | | | |
| Template | 10 | | 50 | | | |
| enzyme | 2 | 10 μl Tag + DV | | | | |

4.50

45 μl / Rx added 5 μl of Mg dif. conc.

To Page N _____

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[Signature]

Date

12/8/94

Invent d by

R corded by

V. Sitarman

Date

12/9/94

Project No. _____

Book No. _____

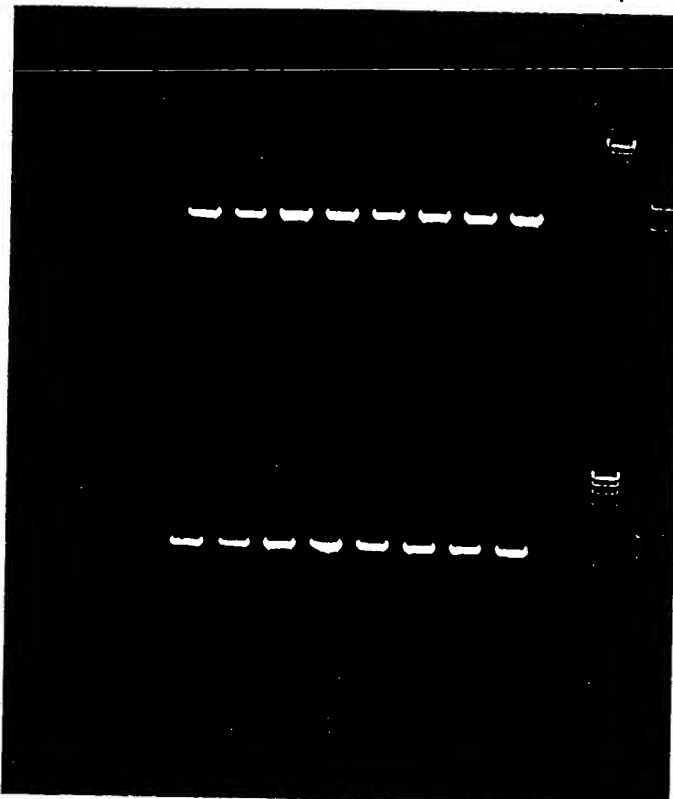
TITLE _____

128 T. J. S.

Fr m Page No. _____

Tag

0 1.5 2 2.5 3 mM Mg



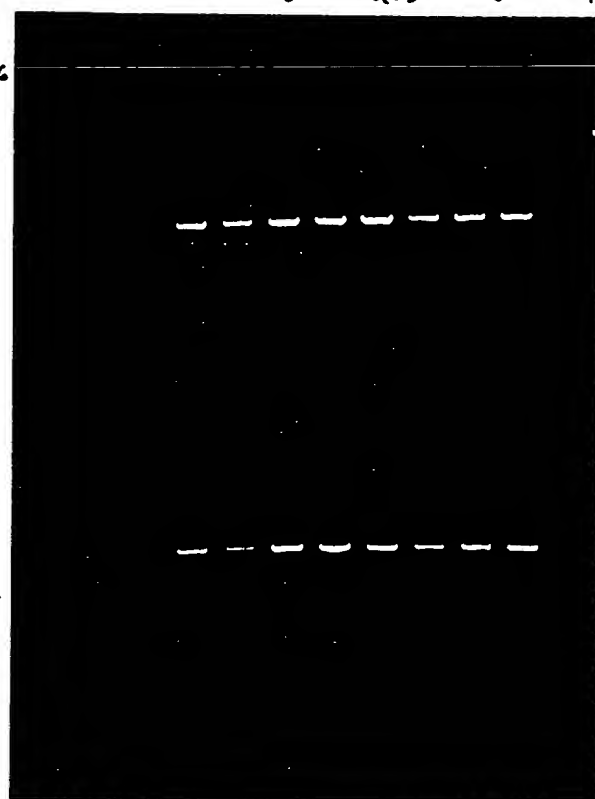
Tag + DV

0 1.5 2 2.5 3 mM

2936
✓
37

*3

*2



2936 x 37

1275 bp product.

Both primers set work with Tag as well as Tag + DV

a bit of mispriming still - has to be gel purified

good range of Mg tolerance

pooled (1) Tag 1.5 mM Rx separately } with *3
 (2) 2.0 } set of
 (3) Tag + DV 1.5 " } primer
 (4) 2.0 " } and phenol ex-
 " ethanol p.p.

To Page

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Ch. Sitarman

12/8/94

Page N. _____

loaded Rx from two tubes (duplicate of same) together in 30 μ l +
made up the volume to 100 μ l 30 μ l

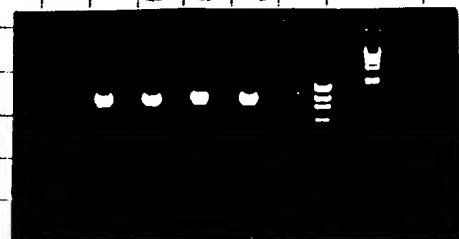
added equal amounts of phenol: chloroform: 2x amygdalin
removed the aqueous phase after a spin of 5' -
phenol extracted again.

added 0.5 volume of 7M ammonium acetate and 1 vol
of ethanol, added also a μ l of Dextran T 500
left at -20° , 1.5 hr

spin down, remove ethanol, washed the pellet with
70% ethanol, spin down, remove the sup.
spin again to remove the residual ethanol
pellet visible, vacuum dried 5'

resuspended in 17 μ l ^{2 TB} - removed 2 μ l for gelling

for 15 μ l added
10.5 " H_2O
3.0 " 10x buffer
1.0 " Afl III (4U/x)
0.5 " Afl II (24U/x)



30 μ l incubated at 37° , 2 hr.

phenol extracted product seems to be around $\sim 150 - 200$ ng/x2
 ~ 75 ng/1x

To Page No. _____

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Date

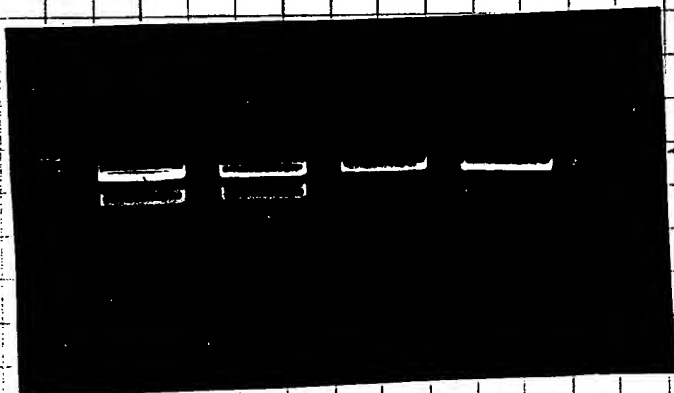
Recorded by

K. Sitarman

12/5/84

From Page No. _____

- 15 μ l of left over phenol chloroform extracted & ethanol pptd. insert & was cut with Hae III and Afl 3 in NEB buffer 4 for 2 hrs at 37°
- Run on 1% gel and transferred to DEAE paper and eluted the fragment in high salt buffer, over the 1M NaCl, 0.1M Tris pH 8.0, 5mM EDTA
- spun down the ethanol buffer, added 50 μ l more & centrifuged, poured the ethanol, ethanol added in \sim 150 μ l \sim 500 μ l in the presence of 1 μ l of Dextrom T. 400.
- left at 70° 2 1/2 hrs, resuspended in 15 μ l after ethanol wash, in TE.



extracted to same as insert!

$$\text{loaded} \sim 75 \text{ ng} \times 15 \mu\text{l} = 1125 \text{ ng} \quad (1275 \text{ bp})$$

$$= 772 \text{ ng} \quad (875 \text{ bp})$$

$$\sim 50\% \text{ recovery} = \sim 386 \text{ ng} / 15 \mu\text{l}$$

$$= \sim 25 \text{ ng} / \lambda$$

To Page _____

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Date

12/18/94

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Date

12/12/94

Recorded by

J. Stamen

Project No. _____

28

Book No. _____ TITLE _____

From Page No. _____

3/8/95 wed

- cftg. the samples for 10-15 min.
- discarded the supernate & rinsed the pellet w/ 70% EtOH
- dried the pellet @ 55°C heat block or @ room temperature
- dissolved the DNA in 50.0 µl TE.

50M Rxn

3/8/95 wed.

Annealing Rxn.

| | + Primer (2899) | - Primer (2899) |
|------------------------|-----------------|-----------------|
| H ₂ O | 3.0 µl | 4.0 µl |
| 5x Buffer | 2.0 µl | 2.0 µl |
| 10 ⁶ 55 DNA | 4.0 µl | 4.0 µl |
| (200mg/4L) oligo | 1.0 µl | — |
| TV | 10.0 µl | 10.0 µl |

Incubated @ 70°C - 75°C for 2 min. (to eliminate non-spf. bin)
" @ 37°C - 40°C for 2 min.

Synthesis Rxn

| | | |
|--|---|----------|
| Annealing Rxn | - | 10.0 µl. |
| 5mL 10x buffer | - | 2.0 µl |
| H ₂ O | - | 6.0 µl |
| T ₄ /T ₇ DNA pol | - | 1.0 µl |
| T ₄ DNA ligation | - | 1.0 µl |
| TV | - | 20.0 µl. |

Incubated @ 37°C for 10 min.

| | | |
|---------------|---|--------|
| Synthesis rxn | - | 2.5 µl |
| TE | - | 8.0 µl |
| loading dye | - | 1.0 µl |

- ran the sample on the gel
- the picture on the next page, # 29.

To Page No

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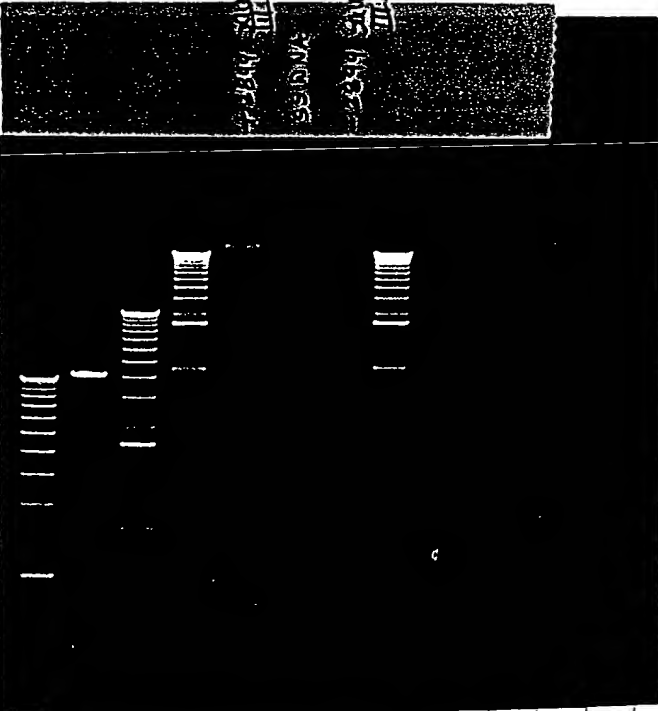
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4/12/95

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4/12/95

Page N _____



(con'd from pg. 28)

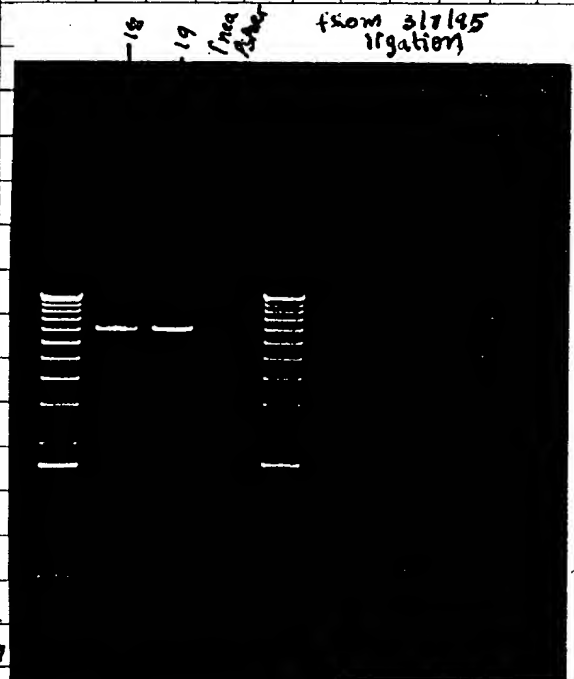
+2899 (w/ primer) oligo forms a ds DNA...

+2899 fragment looks brighter because

Et. Bromide binds to it ^(DNA) better. -2899Primer binds but ~~it~~ does not hold strongly

∴ the DNA fragment looks fainter or light, less Et. Bromide is able to bind.

(con'd on pg. 41)



18, 19, Insert, from 3/17/95 ligation

Ligation from 3/17/95 (pg. 26)H₂O - 8.0 μ l5X buffer - 4.0 μ lmp 18 - 2.0 μ linsert - 4.0 μ lligation - 2.0 μ lTV - 20.0 μ lH₂O - 8.0 μ l5X buffer - 4.0 μ l(vector) mp 19 - 2.0 μ linsert - 4.0 μ lligation - 2.0 μ lTV - 20.0 μ l

- Incubated both samples for 1 hour @ room temp.

 100.0 μ l competent cells
 3.0 μ l DNA

xfection cells.

xfection

10% mp 18 / mp 19

90% mp 18 / mp 19

Control

 ran mp 18 on 3/10
 (used DNA from
 3/10/95 again on
 3/15/95 pg. 32)

To Page No. _____

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4/12/95

From Page No. _____

— 10% mp 18 / mp 19

added { 4.0 mL 2x YT TOP Agar
100.0 μ l X-Gal 4%5.0 μ l IPTG 200 mM (inducer = repressor gives tighter affinity)60.0 μ l lawn cells10.0 μ l xfection cells. (after heat shock for 35 sec.)

— 90% mp 18 & mp 19.

Same way as 10%

— Control

100.0 μ l X-Gal5.0 μ l IPTG60.0 μ l lawn cells.

Date 4/12/95

T Pag N

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Date

4/12/95

Investigated by

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Date

4/12/95

miniprep and digest of
pUC 19 PCR products

Project N _____
B ok No. _____

Exhibit 18
Appl. No. 09/558,421

93

ag No. _____

11-8-94 received plates from Kala S. for pUC19 PCR
with Top + Deep Vent and no Mn^{++} or dNTP bias.
picked 20 white colonies and 2 Blue for 2 ml LB+100 μ g
overnights at 30°C.

12-9-94

miniprep as per (p 41, 4)
using 0.5 ml cells.

resuspended DNA in 50 μ l TE + RNase

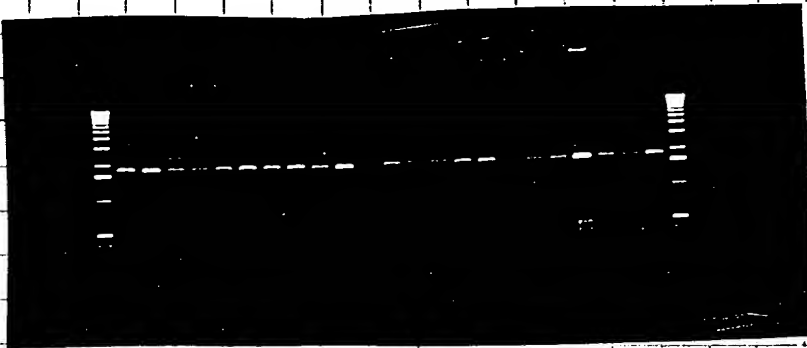
digest each (# 21, 22 ^{are from} the 2 blue colonies)
as follows (tube 23 is 0.2 μ l of 0.8 μ g pUC19)

| | | | | | |
|-------------------------|------------|---|---|---|------|
| 0 | 11.6 | ✓ | ✓ | ✓ | 569 |
| buffer 4 (10x) | 2 | ✓ | ✓ | ✓ | 94 |
| H ₂ O | 5 μ l | | | | |
| RI 10 ^u /l | 0.5 | ✓ | ✓ | ✓ | 23.5 |
| u HI 10 ^u /l | 0.5 | ✓ | | | |
| + II 24 ^u /l | 0.1 | ✓ | ✓ | ✓ | 4.7 |
| (III 7 ^u /l | 0.3 | ✓ | ✓ | | 14.1 |
| | 20 μ l | | | | |

37°C, 2 hours

cocktail for 47 Rn
use 15 μ l Rn
5 μ l DNA

Result:
all are
full length
loc 2



2 loc 2 gives 2 bands

sed & Understood by m ,

Deena Baker

Date

11/6/95

Invented by

Recorded by

To Page No. _____

Date 7-94

12-7-94

Book No._____

From Page No.____

Draw - 2 vials of tag mix - add 22 μ l to the total of 2dCTP-32P-

aliquot 48 μ l to pre-labeled eppendorf's - on ice -
add 1, 2, 4 μ l of diluted enzyme - incubate 10' @
74°C in a heat block - quench w/ 10 μ l of 5%
EDTA - Spot 30 μ l on GFC - Wash

1X 10% TCA 1% Pi
3X 5% TCA
2X 5 fold

Dry and count in LSC
under heat lamp.

| SAM | CFM1 |
|-----|-----------|
| 1 | 1864.00 |
| 2 | 2938.00 |
| 3 | 2940.00 |
| 4 | 940.00 |
| 5 | 1658.00 |
| 6 | 2606.00 |
| 7 | 404.00 |
| 8 | 320.00 |
| 9 | 732.00 |
| 10 | 152.00 |
| 11 | 306.00 |
| 12 | 384.00 |
| 13 | 126.00 |
| 14 | 238.00 |
| 15 | 326.00 |
| 16 | 118.00 |
| 17 | 106.00 |
| 18 | 134.00 |
| 19 | 112220.00 |

6/29/95

To Page M

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Date _____

Invented by

Date _____

May Longo

0/29/53 R c rded by

09/27/45

ge N _____

4/27

| IM | CPM1 |
|----|----------|
| 1 | 6084.00 |
| 2 | 10302.00 |
| 3 | 8286.00 |
| 4 | 3506.00 |
| 5 | 4842.00 |
| 6 | 5272.00 |
| 7 | 1370.00 |
| 8 | 1842.00 |
| 9 | 3392.00 |
| 10 | 182.00 |
| 11 | 85826.00 |
| 12 | 92658.00 |
| 13 | 92494.00 |

.043

7.20 .072

.050

8.5

5.7

5.27

56/12/95

nl - Pool - .075 u/w → ASD Units total -

TIME AVG H#

| | |
|------|------|
| 0.50 | 35.0 |
| 0.50 | 36.0 |
| 0.50 | 40.0 |
| 0.50 | 48.0 |
| 0.50 | 38.0 |
| 0.50 | 42.0 |
| 0.50 | 52.0 |
| 0.50 | 40.0 |
| 0.50 | 40.0 |
| 0.50 | 43.0 |
| 0.50 | 42.0 |
| 0.50 | 44.0 |
| 0.50 | 49.0 |
| 0.50 | 44.0 |
| 0.50 | 42.0 |
| 0.50 | 54.0 |
| 0.50 | 53.0 |
| 0.50 | 46.0 |
| 0.50 | 34.0 |

.08 u/w

ERR

.08 u/w

.063

.08

.071

.055

.075 u/w

56/12/95

SA = 70.1 cpm/pmol
7.01 x 10⁵ cpm/pmol

mf 4/29/95

To Page No. _____

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Date

Invent d by

Date

Mary Lopez

4/29/95

Recorded by

[Signature]

4/27

ge N _____

purpose: To ligate the purified vector + insert and transform with appropriate controls.

Reaction Rx:

Tested

| | | | | | | |
|------------------|-------------|---|------|---------------|---|---------------|
| Vector | ~ 100 ng/μl | = | 1 μl | 1 : 1 " | 1 | Vector alone |
| Insert | ~ 25 ng/μl | = | 4 μl | | 2 | " + real c/po |
| 5x buffer | | = | 4 | | 3 | any purified |
| Ligase Tx | | | 1 | | 4 | Tag 1.5 |
| H ₂ O | | | 10 | | 5 | " 2 |
| 20 μl | | | | at 25°, 3 hr. | 6 | " " 2 |
| | | | | | 7 | " + D.V. 1.5 |
| | | | | | 8 | " " 2 |

transformation using DH5α Max eff. cells.

used 2.5 μl of ligation Mix / transform 50 μl cells

initial volume after adding SOC ~ 500 μl

aliquot 25, 50 & 100 μl of each

control diluted to 1:10 and plated 25, 50 & 100 μl. normal transformation efficiency

Vector only - few blues because of the contamination 1.5 x 10⁹ no whites

Vector + insert - ligated w/o any purification, lots & lots of colonies transformation quite efficient.

Tag + D.V. } 2 mM Mg - didn't work

Tag alone } 1.5 mM Mg

Tag " } Very few colonies in 25 μl + 50 μl
Tag + D.V. } no μl slightly better. In deep well for better than Tag alone, however, are so low to make a call

- all purified gave low efficiency of transformation compared to un " - Vector + insert

Page No. _____

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12/13/94

Dr. Subraman

Page No. _____

3/14/95 TUE

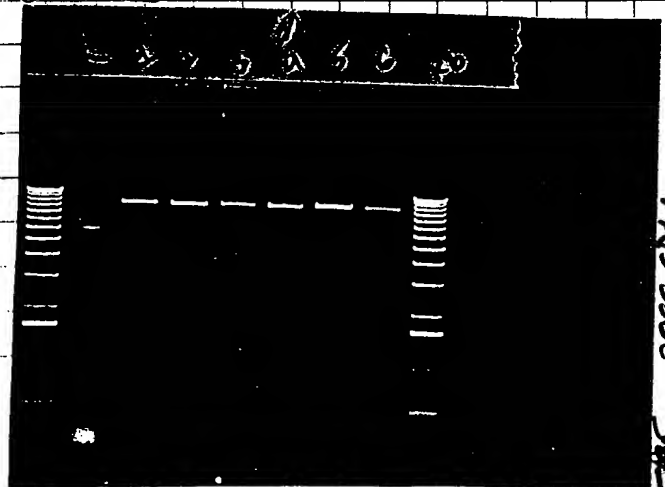
~~Reese~~: miniprep

1.0 ml culture of T-nealmp19 grown for 5 hours @ 37°C in
6 different glass tubes
transferred 1 ml cell to the 6 different labelled eppendorf tubes.
cfg all 6 tubes for 2 min @ room temp.
removed supernate & saved in different tubes
added 100 μ l S1 mixed well
added 200 μ l S2. put the tubes on ice (mixed by inverting)
added 150 μ l 7.5 M NH_4OAc
incubated on ice for 5 min.
cfg for 7 min. @ room temp (4°C) NOTE: cfg in 4°C room was taken away for repair \therefore used @ RT.
transferred supernate (400.0 μ l) to the new 6 labelled tubes
added 800 μ l of EtOH to the 400 μ l of supernate (mixed well)
incubated @ -70°C for 30 min.
cfg for 2 min. @ room temp (discarded supernate)
rinsed w/ 70% EtOH (removed supernate)
added 50.0 μ l TE to the pellet

| | | |
|------------------------------------|-----|----------------|
| H_2O - 7.0 μ l | x 6 | = 42.0 μ l |
| buffer - 2.0 μ l | x 6 | = 12.0 μ l |
| Eco4III - 1.0 μ l | x 6 | = 6.0 μ l |
| TV | | 60.0 μ l |

added 10.0 μ l DNA⁺ to each 6 tubes.

the map is on next page # 32. Fragments
on all 6 tubes are ~~in~~ still present,
y haven't gone into the mutant.
• I tried miniprep again next day.
(started)



d & Understood by me,

Date

Invented by

Date

Polamp

4/12/95

Recorded by

4/12/95

miniprep and digest of
pUC 19 PCR products

ag No. 11-8-94 received plates from Kahan S. for pUC19 PCR
with Top + Deep Vent and no Mn⁺⁺ on JMTV bias.
picked 20 white colonies and 2 Blue for 2 ml LB+100 μ _A
overights at 30°C.

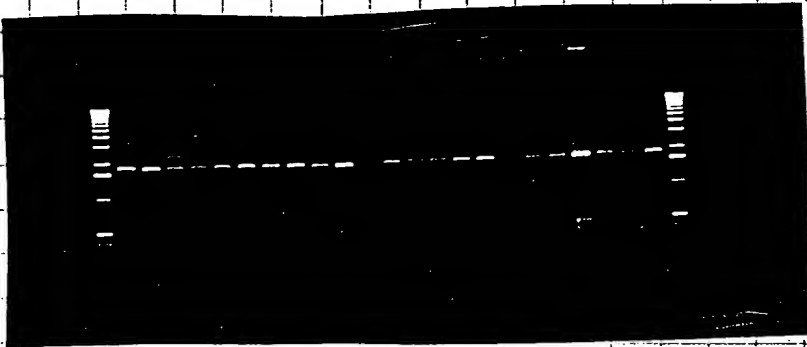
12-9-94
miniprep as per (p 41, 4)
using 0.5 ml cells.

resuspended DNA in 50 μ l TE + RNase

digest each (#21, 22 ^{all from} the 2 blue colonies)
as follows (Tube 23 is 0.2 ml of 0.8 μ l pUC19
569 94 } cocktail for 47 rxn
23.5 } use 15 μ l rxn
5 μ l DNA
37°C, 2 hours

| | | | | | |
|--|------------|---|---|---|------|
| 0 | 11.6 | ✓ | ✓ | ✓ | 569 |
| buffer 4 (10x) | 2 | ✓ | ✓ | ✓ | 94 |
| H ₂ O | 5 μ l | | | | |
| PL 10 ^u /l | 0.5 | ✓ | ✓ | ✓ | 23.5 |
| as H ₂ O 10 ^u /l | 0.5 | ✓ | | | |
| + II 240 μ /l | 0.1 | ✓ | ✓ | ✓ | 4.7 |
| I III 7 μ /l | 0.3 | ✓ | ✓ | | 14.1 |
| | 20 μ l | | | | |

Result:
all are
full length
loc 2



2 loc 2 since 2 bands

sed & Und rstood by m ,

Deena B. B. B.

Date

11/6/95

Invented by

R c rd dby

Date - 7-94

12-9-94

To Pag No. _____

Project No. _____

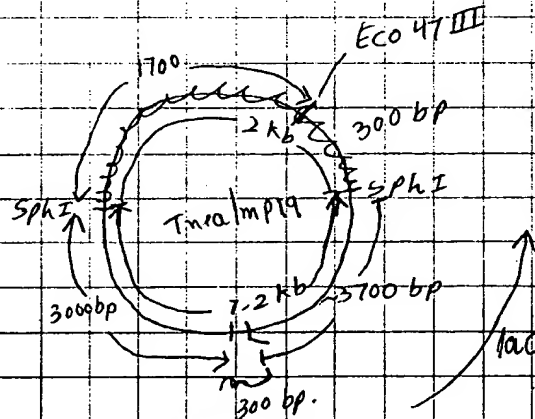
Book No. _____

TITLE _____

32

From Page No. _____

3/15/95 Wed



parent Eco 47 III
~ 8-9 kb
0.3 kb → (most probably won't see fragment because too small & too light)

mutant
4 kb
4.7 kb
0.3 kb

DNA from date 3/10/95



3/10/95
Sph I 700 bp

H₂O = 6.0 μl.
R-6 buffer = 2.0 μl.
mpi8 DNA = 10.0 μl
Sst I/Sph I = 1.0 μl ea.
TV 20.0 μl

H₂O = 6.0 μl.
buffer = 2.0 μl
mpi8 DNA = 10.0 μl
Sst I/Sph I = 1.0 μl ea.
TV 20.0 μl

from pg 29 & ran again on 3/15

arp 4/12/95

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Date

Inventor by

Date

[Signature]

4/12/95

Recorded by

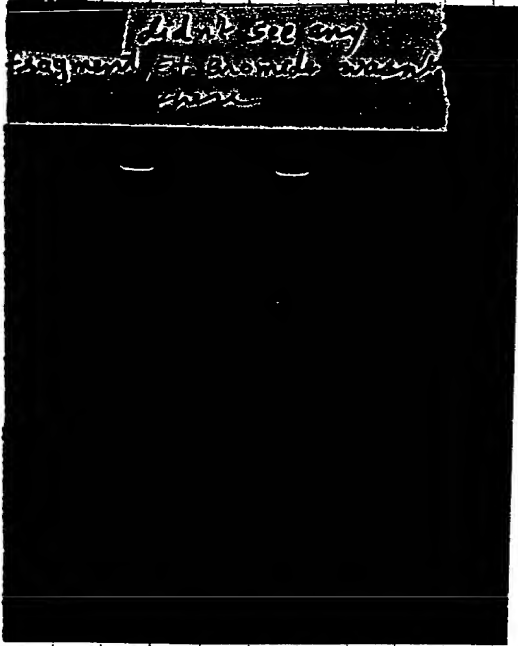
[Signature]

4/12/95

T Page

age No. _____

- Incubated both tubes @ 37°C for 30 min.
- added 2.0 μL loading dye to ea. tube
- ran both on a gel
- took picture



3/15/95 T.nea/mp

1.0 ml T.nea (sph I) /mp 19 +2899 + sau 3AI grown for 5 hours @ 37°C in 10 different glass tubes
 after 5 hours transferred 1.0 ml culture to the 10 labelled eppendorf tubes
 c/f all 10 eppendorf tubes @ room temperature for 2 min.
 removed supernate & saved
 put all 10 tubes w/ pellet & all 10 tubes w/ supernate @ -70°C overnight or until 3/16/95 Thursday.

TE: Brian had to leave @ 4:30 pm & this was a point to stop @.

To Page No. _____

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4/12/95

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Book No. _____

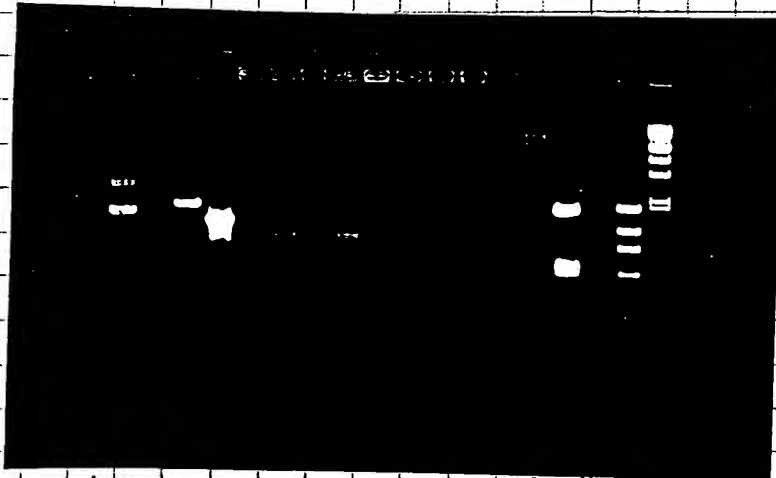
TITLE _____

132

12/13/94

From Page No. _____

- all samples, Vector, inserts, ligation Rxs run on



- ligation seems to be worked - no sign of presence of insert

- excise - linearized puc didn't show up

- purified Vector shows some contamination

- T + D.V 1.5 mM insert barely run

3 purified Vector 1 μ l ~ 100 ng (still has contamination from rest of the vec)

1 insert puc ~ 0.2 μ g

2 linearized puc 2.5 μ g x headed 10 μ l - nothing seen too little.

4 PCR product 2 μ l 12.75 bp.

5 4 purified insert T + D.V 1.5 Rxs

6 4 " 2.0

7 4 " + D.V 1.5

8 4 " + D.V 2 mM

9 8 ligation 9 5 with purified Vector 3

10 6

11 7

12 8

13 " Vector alone

14 " 8 15

15 Vector + rest before purification

To Page

With ssed & Understood by m ,

Date

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Date

Recorded by

12/13/94

K. Sitarman

ge No. _____

since there were 8 of blues and a few whites in 100 ml of
 1) Tag & Tag + 2 vent. reactions plotted once again the
 best of the reactions in a fresh set of plates. 1.5 ml Rx alone

| Tag : | Blue | white | % |
|-------|------|-------|--------|
| | 305 | 4 | |
| | 92 | 1 | 1.26 % |

| Tag + DV : | Blue | white | % |
|------------|------|-------|--------|
| | 161 | 2 | |
| | 201 | 1 | 2.48 % |

| etch only : | Blue | white |
|-------------|------|-------|
| | 64 | — |

Tag + DV is more !!

picked a few blues and

6 whites from Tag

6 " from Tag + DV

same - mini peps.

from next

page.

To Page No. _____

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K. Sivanathan

12/14/94

94

Project No. _____

Book No. _____

TITLE _____

Received 7 clones of T41 pro gene
from AR 12/12/94

From Page No. _____

Follows P115, 6

make 2 ml O/N of each in LB 100 µg/ml Amp 50 µg,
at 30°C

inoculate ~~2 ml~~ ^{0.4} ml into ⁴⁰ 25 ml circle grower
with Amp, Tet (as above)
at 30°C, shaking

started at 8 AM

Ass

11:30 AM

0.296

12:05

0.43

12:30

0.58

induce at 42°C 2 hr starting at 12:45
found OD = 0.87 at 3 PM (store cells pellet)

(mini-preps of 1 ml from O/N)

| | |
|----|------|
| #1 | 1084 |
| 2 | 152 |
| 3 | 106 |
| 4 | 202 |
| 5 | 151 |
| 6 | 107H |
| 7 | 109 |

see P 157 -
will try induction at
42°C, 15' and
37°C 40 min

digest with Alu AfI III, AatII
and EcoRI in NEB buffer 4
(see P 93) 2 hr, 37°C

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Date

Invented by

Date

To Page

Domena Bolero

1/1/95

Recorded by

12/12/94

12/13/94

From Page No.____

Purpose: To make more ligation reactions at a different ratio of Vector: insert + transform.

| | | |
|------------------|-----|-----------|
| Vector | 0.5 | } n-1 + 3 |
| insert | 6.0 | |
| 5x buffer | 4.0 | |
| T4 ligase | 1.0 | |
| H ₂ O | 8.5 | |

Same the same for all Res

- Vector alone

~ Tag

- Tag + Deep Vent }

20 μ l at 25° 3 hrs

- used 2.5 μ l of each reaction to transform DH5 α n efficiency cells.

- included untreated pbc (monomer) Control

- normal protocol.

ligation box

Vector only : receipt - 51

Tag

| | | |
|------------|---|------|
| 25 μ l | - | 1.50 |
|------------|---|------|

| | | | | |
|----|--|--|---|-----|
| 50 | | | - | 352 |
|----|--|--|---|-----|

100 - 504

| | |
|-----|---|
| 1.8 | 2 |
|-----|---|

| |
|------|
| 1006 |
|------|

Tag + DV

| | | |
|----|--|-----|
| 25 | | 124 |
|----|--|-----|

| | | |
|----|--|-----|
| 50 | | 274 |
|----|--|-----|

| | |
|-----|-----|
| 100 | 487 |
|-----|-----|

0.72

885-

In this set T+D.V. : seems to be still a very hard count.

Diagram illustrating the components of a vector:

- 1. Vector
- 2. Tag
- 3. Vector + rest
- 4. Tag + DV

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To Page No.

12/12/94

Project No. _____

Book No. _____ TITLE _____

34

From Page No. _____

3/16/95 Thurs.

con'd from page 33 3/15/95 wed. MINIPREP

- took the pellet out from -70°C (10 eppendorf tubes)
- added 100 μL S1 mixed well
- added 200 μL S2 put all 10 tubes on ice. mixed
- added 150 μL 1.5 M NH_4OAc
- incubated on ice for 5 min.
- cfg all 10 tubes for 5 min. @ room temp. (4°C)
- transferred 400 μL of supernate to the new 10 labelled tubes
- added 800 μL EtOH. Mixed well
- incubated all 10 tubes for 30 min. @ -70°C .
- cfg & discard for 2 min. @ room temp.
- discarded supernate & washed pellet with 70% EtOH.
- added 50 μL TE to all 10 tubes w/ pellet

| | tubes | |
|----------------------|-------------------|--------------------------------|
| H_2O | 1.0 μL | $\times 10 = 10.0 \mu\text{L}$ |
| buffer | 2.0 μL | $\times 10 = 20.0 \mu\text{L}$ |
| ECO 47III | 1.0 μL | $\times 10 = 10.0 \mu\text{L}$ |
| | T.V | $= 100.0 \mu\text{L}$ |

- added 10.0 μL from T.V to all other 9 tubes
- added 10.0 μL DNA to each 10 tubes

- incubated @ 37°C for 30 min.
- added 2 μL loading dye
- ran all 10 samples on a gel for 1 hour @ 190 V
- took a picture

picture on pg 35

To Page N

Witness d & Understood by me,

Dat

4/12/95

Invented by

Recorded by

Dat

4/12/95

ag No. _____

parent & mutant should look like
[ECO 47 III]



ECO 47
3/16/95
10/1/95

parent

mutant

8.9 kb

4.5 kb

0.3 kb

4.4 kb

0.3 kb

may probably be too light to see

NOTE: In this we could see parent & some mutant. mutant is seen on # 5, 6, 7, 8

PO

To Page No. _____

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Date

[Signature]

4/12/95

Recorded by

[Signature]

4/12/95

Extract TFI cells and heat
treat as per P. 115, 6

Project No. _____

Book No. _____

Results on P 97

Exhibit 21

Appl. No. 09/558,421

15

9 N - with 1ml Tag ext buffer (P117, 3) (+ PMST and 15ME

Pol array

maxA

(for 9 x $V_p = 10.1$ Rxn)
(for 27 Rxns)

Red is
3-23-95
cocktail

x TFI Reaction
(equivalent -
mM Tris pH 9
Ammonium

45 μ l

✓ ✓

131 67.5

25 mM
NTPs 10 mM each
H₂O
2 CDP
3.7 mg/ml

72

✓ ✓

54

(50 mM) $V_p = 2$ mM
200 μ mol

18

✓ ✓

27

597

✓ ✓

870

10.5

✓ ✓

4

122

✓ ✓

183

V_p 855

(use 95 μ l / 100 μ l Rxn)

$V_p = 1215$

use 45 μ l / 50 μ l
Rxn

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|-------------|---|---|---|---|---|---|-----|-----|
| 95 μ l | — | — | — | — | — | — | — | — |
| 202 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 4 | | | | | | | | |
| H | | | | | | | | |
| 0.1 nM | | | | | | | | |
| 100 μ l | | | | | | | | |
| | | | | | | | 0.5 | 4.5 |

72°C remove 20 μ l to 5 μ l 0.2 M EDTA at 15, 30, 60 min
Results on P 97

note - all clones have thermostable activity - eg #107 is ~0.14 μ l
Tag is ~25 μ l (P36) and 40 mg/ml in Fr I (P 37)

afford on Fr I's

To Page No. _____

| | | | |
|--------------------------------------|-----------------|-----------------|------------------|
| d & Understood by m ,
evan Boring | Date
11/6/95 | Invented by
 | Date
12-14-95 |
| Rec'd by | | | |

Page No. _____

mini pigged 2 blues
 Top 6 white
 6 "

12/14/94

Plated again the left ones from 12/13/94 transformation R₀

| <u>h</u> | <u>co</u> | | | |
|----------|-----------|-----|---|--|
| 35 | - | | | |
| 528 | 6 | | | |
| 437 | 4 | 1.1 | % | |
| 567 | 7 | | | |
| 1532 | 17 | | | |
| 569 | 4 | | | |
| 502 | 3 | | | |
| 579 | 5 | 0.7 | % | |
| 1650 | 12 | | | |

average }
 2 } 1.4 %
 2 day }

ult: Once again # of whites are less than both
 of & Tag + DV give ~ 1 - 2% mutants + these
 are not satisfactory to make a call. Tag: T+DV = 2:1!
 To Page No. _____

ed & Understood by me,

Dat

Inv nt d by

Dat

Rec rded by

A. Sitarasman

12/15/94

Results on H1 pol activity from P95

Project N. _____ Exhibit 22
Appl. No. 09/558,421

Book No. _____

97

| SAM | CPM1 | pmol | u/ml |
|------|-----------|------|--------------|
| 201 | 10297.00 | 473 | 0.037 |
| 102 | 22380.00 | 1028 | |
| | 42363.00 | 1946 | |
| | 25336.00 | 1164 | 0.09 |
| 106 | 44240.00 | 2033 | |
| | 82378.00 | 3786 | |
| | 36103.00 | 1659 | 0.129 |
| 107 | 58201.00 | 2675 | |
| | 90720.00 | 4169 | |
| | 39104.00 | 1797 | 0.144/ml |
| 108 | 57842.00 | 2657 | |
| | 106183.00 | 4880 | |
| | 4229.00 | 194 | 0.015 |
| 109 | 8062.00 | 370 | |
| | 17941.00 | 824 | |
| | 20144.00 | 926 | |
| 151 | 37486.00 | 1722 | 0.134 |
| | 65420.00 | 3006 | |
| | 23430.00 | 1077 | 0.083 |
| 152 | 43025.00 | 1977 | |
| | 71820.00 | 3301 | |
| | 37673.00 | 1731 | 0.7 u/ml |
| Tf1 | 63089.00 | 2899 | |
| 05 u | 99545.00 | 4575 | |
| 25 | 871.00 | BKED | |
| 22 | 109915.00 | 62 | 10 u/ml/pmol |

| 5 u | Brodyfrut | I' | Capto 5'900 |
|-----|-----------|-------|-------------|
| | | mg/ml | u/mg |
| 201 | 204 | 0.44 | 83 |
| 106 | .225 | 0.49 | 185 |
| 107 | .251 | 0.55 | 235 |
| 108 | .220 | 0.48 | 291 u/m |
| 109 | .230 | 0.51 | 29 |
| 151 | .23 | 0.51 | 265 |
| 152 | .20 | 0.44 | 190 |

note: get ~ 10,000 - 20,000 u/mg from TTQ Tag (Fr I')
clone, eg see P104-105, 7 and P36-37 (this book)

~~if ~ 5 mg/ml~~

~~still only ~~~

get ~ 200 unit/mg in I'

~~resp~~ so ~ 100x less activity here

To Page No. _____

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Date

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Date

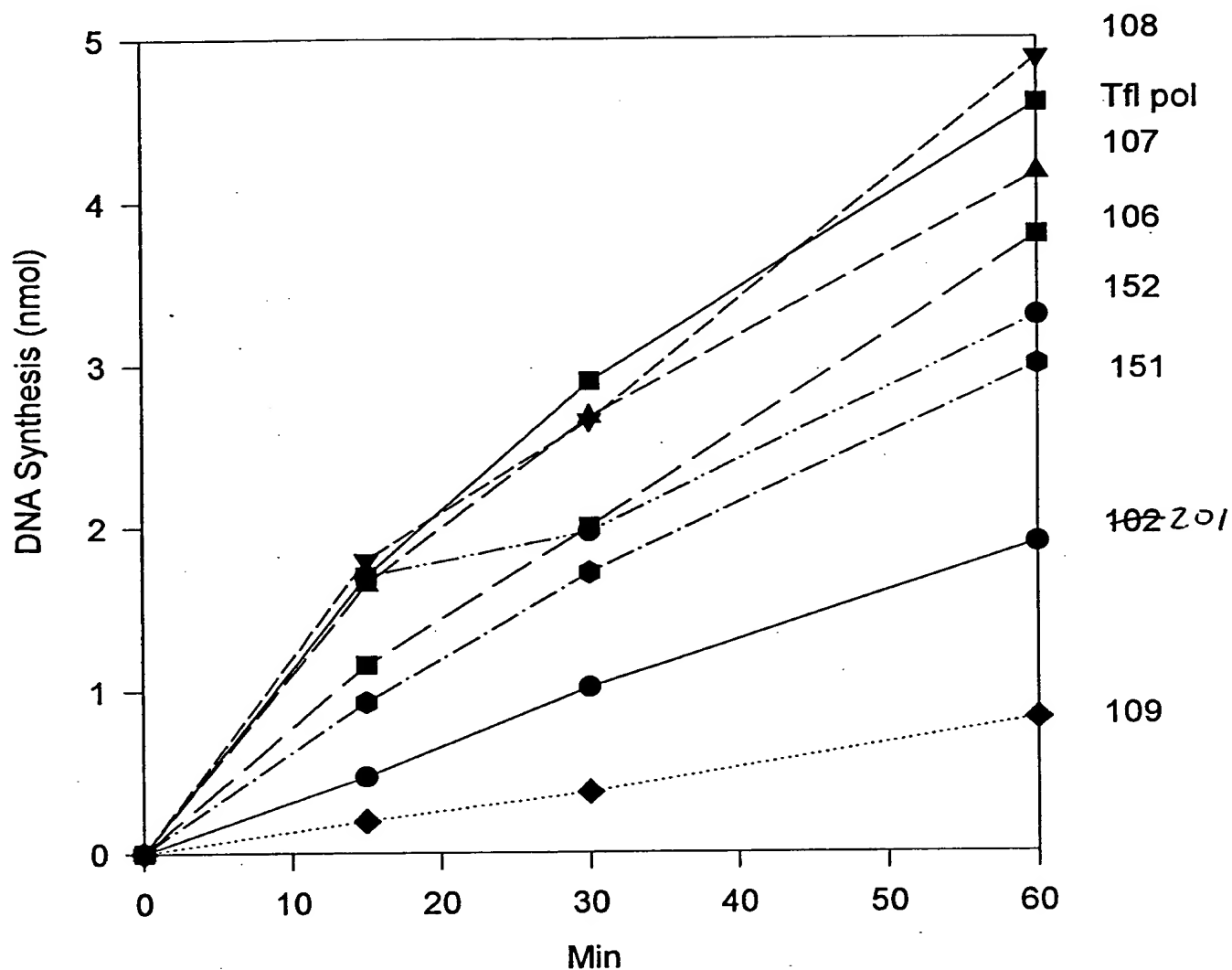
even a Polan

1/6/95

Recorded by

17-15-94

From Page No. ____

Tfl clones

To Page I

Witnessed & Understood by me,

Deena Golani

Date

1/6/95

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Recorded by

Date

12/15/94

End label 50 mer for test
of STMP incorp opposite template U

Project N. _____

Book No. _____

99

Page No. _____ End label as per ~~P128~~ P128, 6 (and P132, 6)

ligo 733 (30mer)

69.4 pmol/l

↓ dil 1/69.4

= 1 pmol/l

5 X kinase buffer

PNK

32P ATP

H₂O

1 µl

2

21

40 µl

↓ 37°C, 30' → 55°C, 5'

40 µl

15 µl

4 µl

73 µl

132 66 µl

10 pmol

100 pmol (30 10X
excess of
6780)

(.076 pmol of)
32P 733

itled.seq Length:85 Tue Nov 29 10:10:41 1994

er-Lower Dimers

r positions: untitled:1U85 untitled:61L18

/Lower: the most stable 3'-dimer: 2 bp, -3.1 kcal/mol

AAAAGTCACCTGCATCAGCAATAATTGTATATTGTGGAGACCCCTGGAACCTATAGGAATTAATGAAGGAGAATTCGGGTC
3' ATTACTTCCTCTT

/Lower: the most stable 3'-dimer: 18 bp, -32.9 kcal/mol

AAAAGTCACCTGCATCAGCAATAATTGTATATTGTGGAGACCCCTGGAACCTATAGGAATTAATGAAGGAGAATTCGGGTC
3' ATTACTTCCTCTTAAGGC 5'

/Lower: the most stable dimer overall: 18 bp, -32.9 kcal/mol

AAAAGTCACCTGCATCAGCAATAATTGTATATTGTGGAGACCCCTGGAACCTATAGGAATTAATGAAGGAGAATTCGGGTC
3' ATTACTTCCTCTTAAGGC 5'

2 U₂

32P 733

To Page No. _____

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Date

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Dat

erica Polamp

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12/15/94

Project No. _____

Book No. _____

TITLE _____

136

12/14/94

From Page No. _____

Purpose: To try *Agarose* in balance & in transformation & whether we get better &.

L 1 = Tag 1 U (200 μ M each nucleotide) 1

L 4 = Tag 4 U " " 2

Tag: H 1 = " 1 U 200 μ M dA & sub 1 mM 3

H 4 = " 4 U " " 4

L-D 1 = Tag - DV 1 (200)

L-D 4 = " 4 " "

H-D 1 = " 1 (200 + 1 x 3)

H-D 4 = " 4 " "

Supernatant of each reaction pooled together, ethanol pptd after a phenol chloroform extraction.

Resuspended in 15 μ l reaction TE sub with Afl III and Aat II in NEB buffer overnight at 37°.

2 μ l of each run on gel to see the digestion is com.

Even though *Agarose* said there is enough product in PCR & some of them didn't show up on the gel after all the purification steps.

Since there is not much time to gel purify the fragments whole reaction as such, was used in the ligation reaction.

2 10 μ l of it.

To Page No. _____

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Date

Initiated by

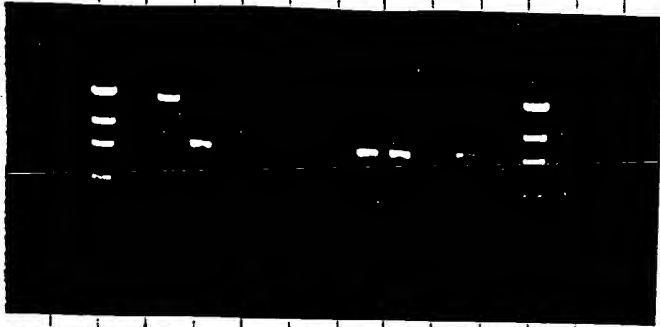
Date

Recorded by

12/15/94

Dr. Shivanian

ag N _____



Vector Tag Tag + SV
Ry

Vector 1 µl
insert 10 µl
ligase 1 µl
76
5x buffer 4 µl

200 µl

at 25°, 3 hrs.

transformed all 10 (1-10 above) & control insert minus pvc.

ligation

1. Vector only
2. Vector + rest
3. Tag 2.1
4. 1.4
5. 1.1
6. 1.4
7. T+SV 1.3
8. 1.3
9. 1.1
10. 1.4

10 µl of

Restriction

don't know exactly how much

insert is there

Read & Understood by me,

Date

12/16/94

Inv. nted by

R. corded by

St. Blacaman

Dat

12/16/94

To Page No. _____

Project No. _____

Book No. _____ TITLE _____

100

From Page No. _____

(1) (2) (3) (4) (5) (6) (7) (8) (9) (10)

32P 733: 678

5 —————→ V

P.99

5 —————→ V

10x Ultima

10x PCR buffer (Tag)

5 5 5 5 5 —————→ V

1x 20x buffer

2.5 5 —————→ V

10x Vent buffer

5 5 —————→ V

10x Pfu II

4 dNTPs 10mM each

Ultima 6 u/l

Tag 3 u/l EKBT (P509)

Taq 5 u/l 11-2-34

Tfi 1 u/l

Tth (P2E) 2.5 u/l

Vent 2 u/l

Deep Vent 2 u/l

Pfu 2.5 u/l

D10K 20 u/l

25mM MgCl₂H₂O

34.6 34.2 34.5 35 35 37.8 37.8 37 34.7 35 V

70°C remove 10 µl to 5 µl cycle seq stop solution
at 1, 10, 20

To Page N

Witnessed & Understood by me,

Deena Polaris

Dat

1/6/95

Invented by

Rec rd d by

Dat

12/16/94

ag N _____

10x ~~alt~~ ^{alt} ~~prim~~ ^{prim} 733 total / 50 μ l (7.6 nM primer total)

10 mM Tris pH 8.8, 10 mM KCl, 0.02% Tween 20

PCR buffer (BRL) # Y0202P, 20 mM Tris pH 8.8, 50 mM KCl

(200 μ M each dNTP)

units pol (~0.125 pmol pol molecules)

$\frac{0.0}{0.0} \sim 3^{32}\text{P}733 / 1$ pol molecules

KPFA at 1X = 20 mM Tris pH 8.8, 10 mM KCl, 10 mM $(\text{NH}_4)_2\text{SO}_4$, 2 mM MgSO_4 , 1% Triton X-100

DTA = 20 mM Tris pH 7.5, 20 mM KCl, 10 mM $(\text{NH}_4)_2\text{SO}_4$

To Page No. _____

Issued & Understood by me,

Date

Invented by

Date

Sara Polansky

1/6/95

Recorded by

12/16/94

Project No. _____

Book No. _____

TITLE _____

138 12/16/94

| From Page No. | Tag | B | W | % | Tag + DV | B | W |
|---------------|-----|-----|---|-----|----------|-----|---|
| L | 1 | 837 | 8 | 1 | LD 1 | 984 | 2 |
| L | 4 | 777 | 7 | 1.2 | LD 4 | 599 | 7 |
| H | 1 | 782 | 7 | 0.9 | HD 1 | 732 | 8 |
| H | 4 | 920 | 4 | 0.4 | HD 4 | 691 | 3 |

nothing to great about!

| | | | | | | |
|-----|-----|-----|---|------|-----|---|
| L 1 | 25 | 156 | 0 | LD 1 | 206 | 0 |
| | 50 | 260 | 1 | | 341 | 1 |
| | 100 | 421 | 7 | | 437 | 1 |

| | | | | | | |
|-----|-----|-----|---|------|-----|---|
| L 4 | 25 | | | LD 4 | 101 | 2 |
| | 50 | 51 | - | | 208 | 2 |
| | 100 | 119 | 3 | | 290 | 6 |
| | 150 | 251 | 2 | | | |
| | 200 | 356 | 4 | | | |

| | | | | | | | |
|-----|-----|-----|---|------|-----|-----|---|
| H 1 | 25 | | | HD 1 | 25 | 95 | - |
| | 50 | 92 | 2 | | 50 | 216 | 2 |
| | 100 | 119 | 2 | | 100 | 421 | 6 |
| | 150 | 216 | 2 | | | | |
| | 200 | 355 | 1 | | | | |

| | | | | | | | |
|-----|-----|-----|---|------|-----|-----|---|
| H 4 | 50 | 69 | - | HD 4 | 25 | 113 | - |
| | 100 | 128 | - | | 50 | 248 | 1 |
| | 150 | 315 | - | | 100 | 330 | 2 |
| | 200 | 408 | 4 | | | | |

To Page No.

Witnessed & Understood by me,

Date

12/16/94

Invented by

Recorded by

Dr. Sitae man


Date

12/16/94

TITLE

Phylogenetic tree showing the three domains of life: Eubacteria, Archae, and Eukarya. The tree is rooted at the bottom with a scale from 0 to 1.0 in increments of 0.2. The Eubacteria branch includes Ultime, rTag, Tne, Tfi, rTth, Vent, and Deep Vent. The Archae branch includes Pfu and D.Tok. The Eukarya branch is shown as a single line extending to the right.

— primer

$3'P$  $3'$ UUAUACGACGUCCAC

750-50

To Page 1

Deeraa Polay

2. 16/25

Recorded by

12/17/54

date: 1/8/95 where only (-) Mn

Project No. _____

Book No. _____

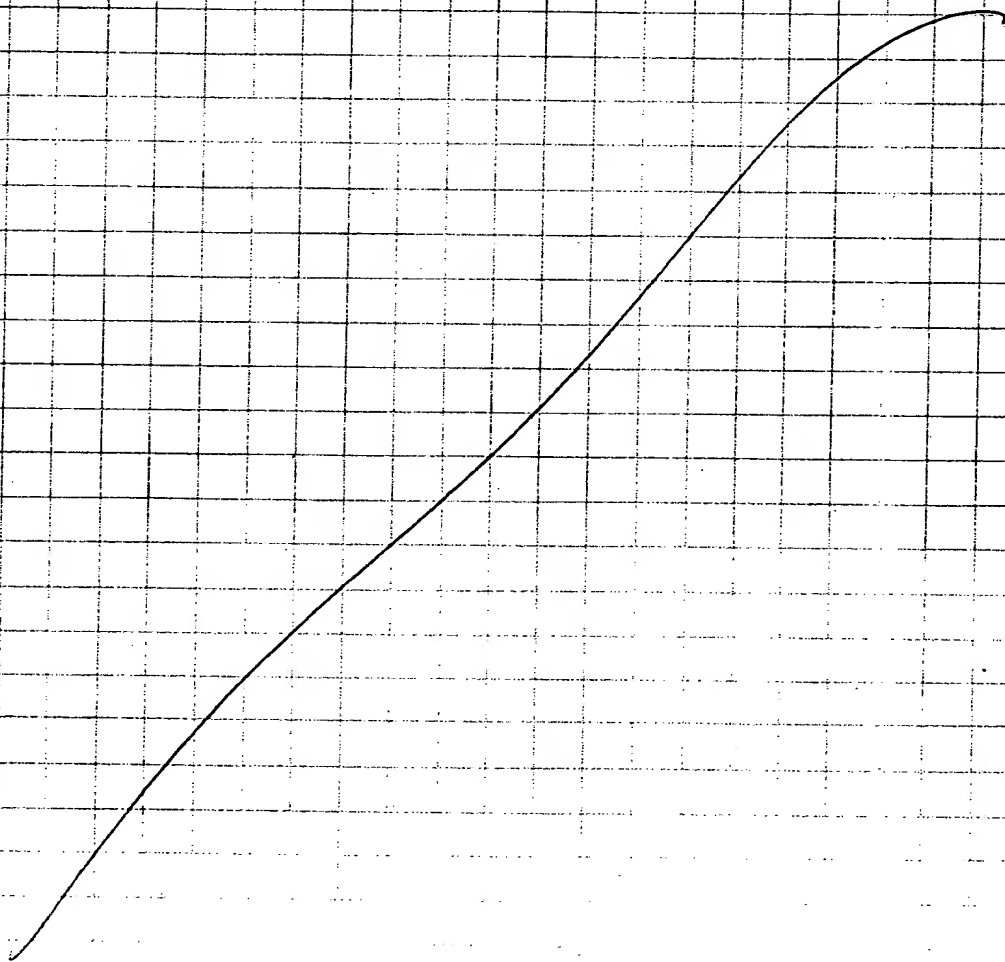
103

ag N - condition same colonies

this is to reconfirm result of p93 that auxotrophic
strain on pUC 19 gives full length lac in white colonies

plates are all 0/5 = no Mn⁺⁺ and 5- μ Tag
pick 5 into 2 ml LB + 100 μ g 1 ml LB
30°C 0/N

Didn't complete this experiment



Read & Understood by me,

Michaela Polak

Date

2/16/95

Invented by

Recorded by

To Page No. _____

Date

1-10-95

Pick 3 wheels from H20012. experiments
done 1/8/95 where only (-) Mn

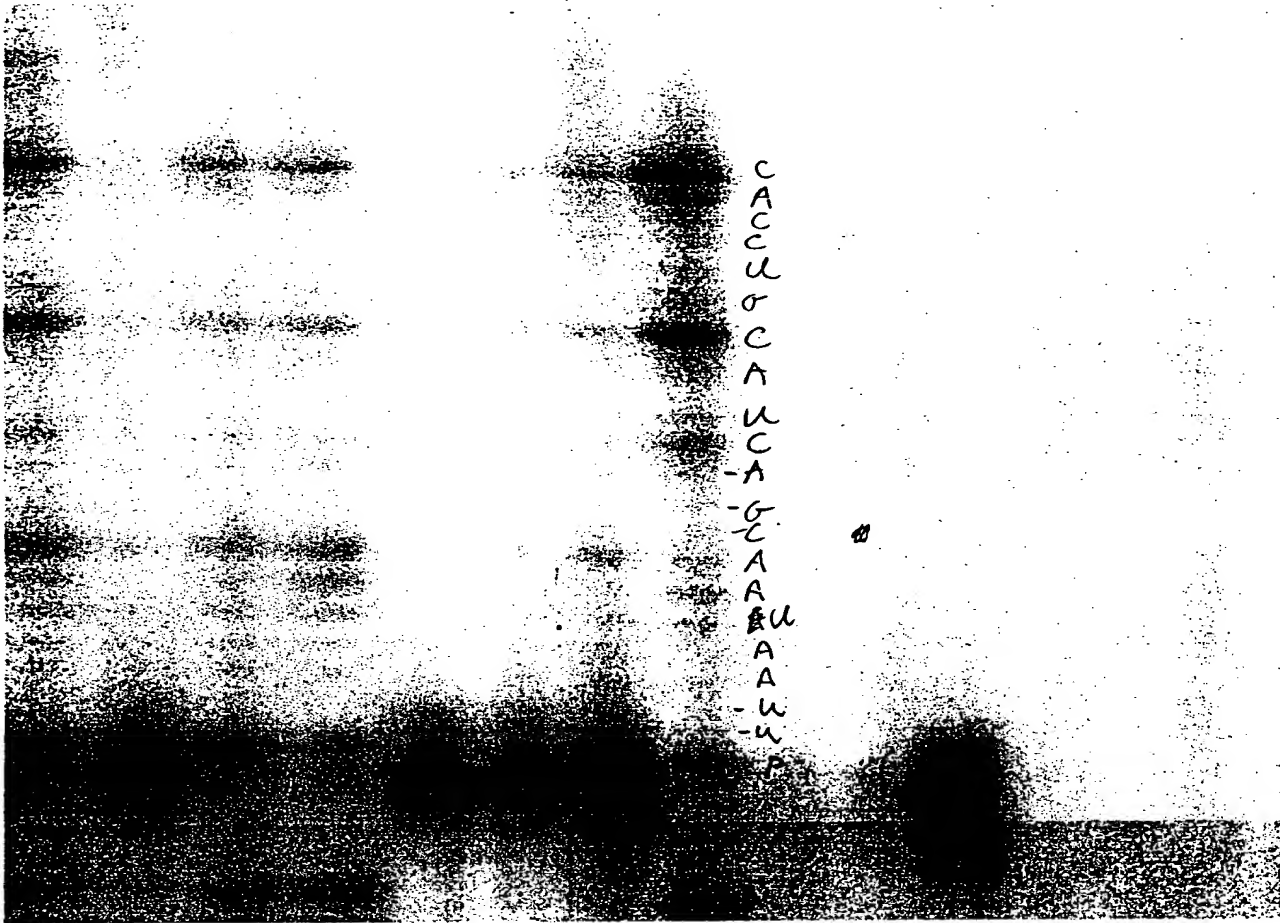
Project N _____

Book No. _____

103

ge N _____ conducting game colonies

min
the colonies
Tag



2.00 699.88

2.00x Counts

- 12/17/94 - 10:49 pm

To Page No. _____

d & Underst od by m ,

Date

2/16/95

Invented by

Recorded by

Date

1-10-95

saal a Polap

ag No. _____

purpose: To minimize the overnight cultures to which
all solutions from above

1.5 ml D

pellet resuspended in 0.1 ml of Resuspension buffer

25 mM Tris, 8.0

50 " EDTA

1% glucose

autoclaved + stored at 4°

added 0.2 ml of NaOH / 500 = 0.2 M NaOH

on in 15

1% SDS

prepared fresh

added 0.15 ml of 7.5 M Ammonio acetate Mix + incubate 15 min

15' RT filtered + not filtered

sup + 0.9 ml of 95% ethanol Mixed well 15'

white washed with 70% ethanol 15ml w/o mixing 15'

again Vac dried 5'

resuspended in 25 μl (100 ng/1 μl) of TE

used 8 μl from each prep for digestion

Reaction buffer 10x NEB 4, Afl III, Aat II & EcoRI

90 μl

5

2

3 μl

rest H₂O, Volume to 192 μl

added 12 μl / Rx digested at 37° , 3 hrs, stored at 4°

To Page No. _____

Issued & Understood by me,

Date

12/16/94

Invented by

Recorded by

J. S. S. S. S.

Date

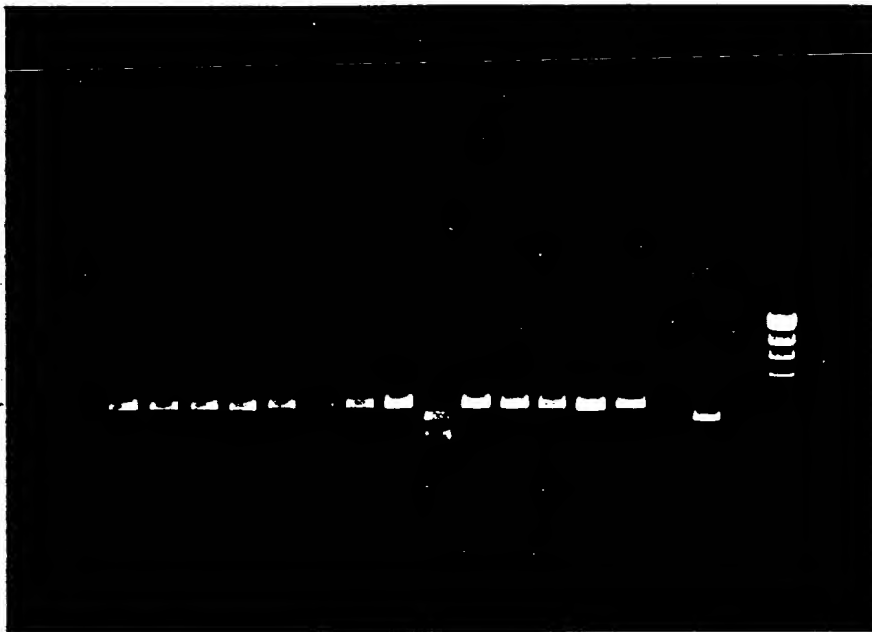
12/16/94

Project No. _____

Book No. _____

TITLE _____

From Page No. _____



Vector

B W. from Tag W from T+DV

Even Palmer didn't get restricted why?

T Pag N.

Witnessed & Understood by me,

Date

Invent d by

Dat

Record d by

12/22/94

Dr. Sitarman

PMCA Revisited:

Tag N .

Purpose: To repeat the exercise again with pmca for fidelity

Just :: to titrate the amount of template I used 100 - 200 pg / 35 cycles / 3 step 3' extension - but of sequencing was obvious. Today has used earlier has template / 30 cycles / 2 step has of other products 5' extension

so tried 0, 10, 25, 50, 100 & 200 pg of starting template / 5' extension / 2 step

both 10 & 20 of enzyme product yield is negligible - but with 20 of Tag quite good product yield was obtained

so tried with both 10 & 20 of Tag alone

To start with, in this expt Tag, D1 wasn't included

Conditions:

10x buffer K.T.

94°, 30"

200 μ M dNTP

.4 μ M primers (new-bw)

30 (94°, 30", 60", 5')

2 mM Mg

Template 0 - 200 pg

enzyme 1 & 20.

- prepared a premix 12x 45 μ l

7 tubes: 1 - 12 10

13 - 24 20

- added 5 μ l of different amount of template

To Page No. _____

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Date

1/9/95

Invented by

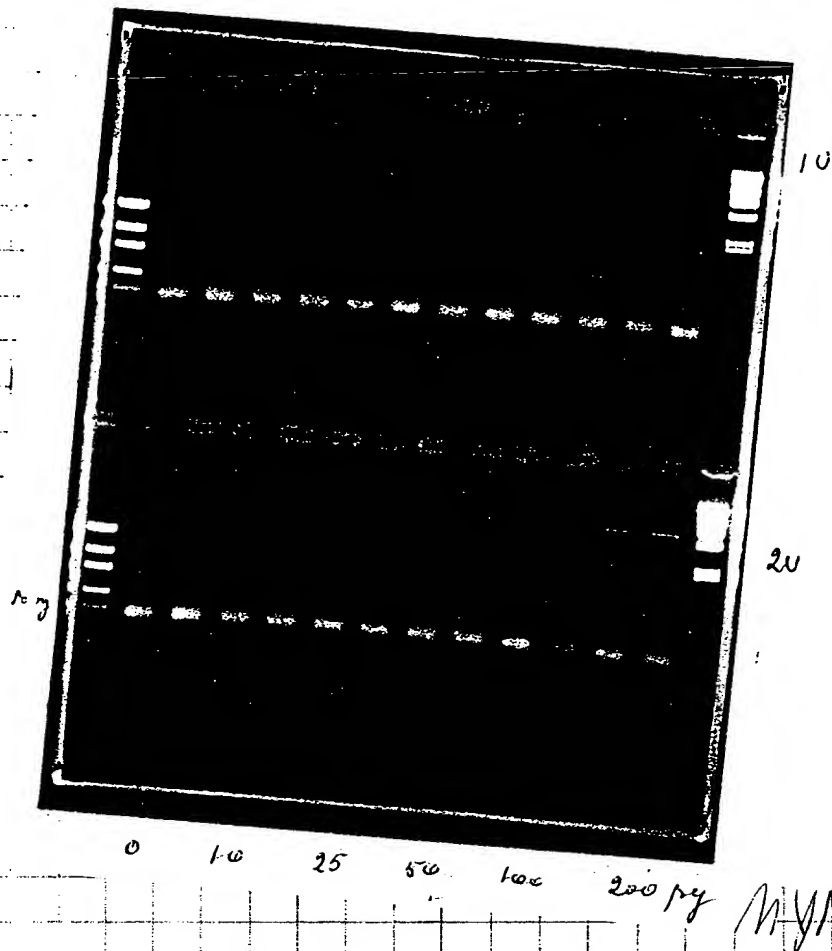
Date

Record d by

K. W. Brennan

12/19/94

From Page No. _____

TagResult :

200 pg / 10 enzyme ✓

25 pg up / 20 produced
obtained but
rather very
yield even with
200 pg of starting
template.

~ 5 - 10 ng / spl

= 31.25 - 62.5 ng / $\frac{1}{2}$
from 200 pg temp

~ 300 fold ampl

~ 8 doublings !

* Try 50 pg } 20 200 pg - 10

- cycles increased to 35 atleast

- go up to 50, 60 -

- cycling regime looks ok - gives cleaner product.

no mispriming seen.

To Page N

Witnessed & Understood by me,

Date

1/9/95

Invented by

Recorded by

Dr. Sitarman

Date

12/20/94

104

Project No. _____

Book No. _____

TITLE

SDS of rTag & Native Tag pur

From Page No. _____

repeat of 10-3-94 with high amounts of Native Tag

TCAppt. of Native Tag (see P96,7)

Native Tag 5 μ /pl
lot # EPD 404
total units
H₂O
15% TCA (ice cold)

| (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) |
|-----|----------------|-----|-------|-----|-------------|-----|-------------|
| | 16 | | 32 | | 64 | | 96 |
| | (80) | | (160) | | (320) | | (600) |
| | 184 | | 168 | | 136 μ l | | 104 μ l |
| | 200 | | | | | | |
| | $\Sigma = 400$ | | | | | | |

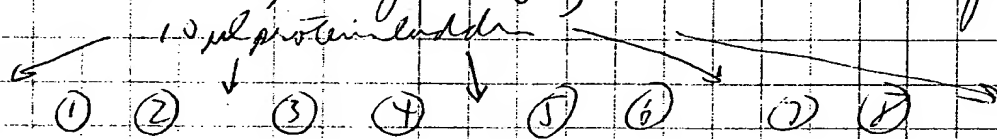
ice 30'
microfuge 10'
resuspended in acetone (-20°C) stored at
microfuge 15', dried and super
dry 37°C
resuspended in 50 μ l 1X sample buffer

rTag EKRT1 lot #
dilute 1/4 in 1X crack
(now its 80% μ l)

1X sample buffer

| | | | |
|-----------|----|----|----|
| 1 μ l | 2 | 4 | 46 |
| 50 | 48 | 46 | 44 |

Load all 50 μ l after 5', 90°C as follows



gel same as P140, 6

1.5 min spacer
1X wells

start 10:10 AM at 28 mA (= 72 V)
(maintain ~ 30 mA constant)

same as P148, 6

stopped gel at 3:20 5 hr 10 min total time

To Page N

Witnessed & Understood by me,

Deena Pokors

Date

2/16/95

Invented by

Recorded by

Date

1-11-95

106

Project No. _____

Book No. _____

TITLE

miniups for Aeyou's plates
of 1-10.95 0, .05, 0.1 mM Mn

From Page No. _____

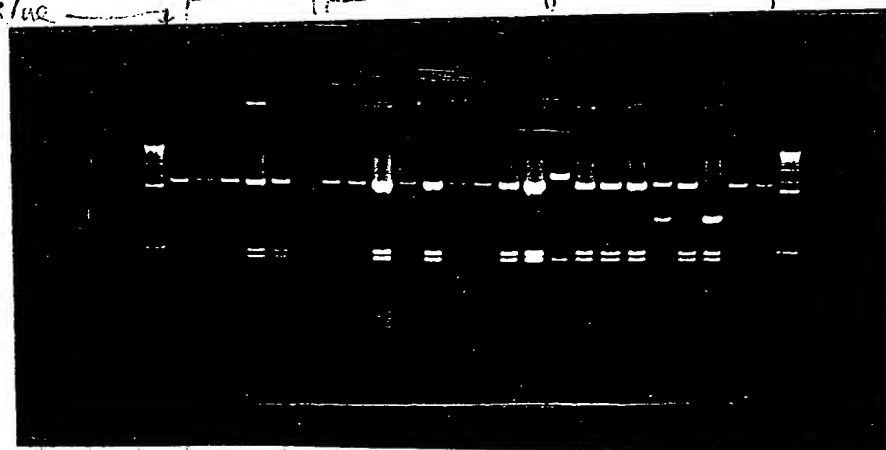
for puc PCR

grow O/N, 2 ml carb grow, 10⁶ pg/ml

| | | | | |
|-------------------------|------|-------|--------|-----|
| mM Mn Cl ₂ = | 0 | 1 | Blue | (1) |
| | 0 | 2-6 | whites | (5) |
| | 0.05 | 7-15 | whites | (9) |
| | 0.1 | 16-24 | whites | 9 |

best same as P.93, 2 hr 37°C Aat II, AFI III, Ew R, NEB by

Mn++ 0 0.05 mM 0.1 mM
Blue



↑
- Aat II
only
410bp
AFI/R1
band is present

↑
- R1 m#25
R1 is missing
875
Aat ↑ AFI
R1

Results:

for 0.1 mM Mn Cl₂ 3 of 9 are rearrangements;
2 are in loc region, one is in vector
0 and 0.05 mM Mn are 9/9 full length

To Page N

Witnessed & Understood by me,

Deerana Polano

Date

2/16/95

Invented by

Recorded by

Date

1-12-94

Page No. _____

Purpose: Amplification of pMC9 with 20 μ g Tag and different amount of Deep Vent.

prepared mixture with 20 μ g Tag w/o any Deep Vent.

added dif. amount of Deep Vent, done in duplicate.

| Tag | D.V | μ l |
|-----|------|---------|
| 20 | 5 | 2.5 |
| | 2 | 1 |
| | 1 | 0.5 |
| | .5 | 0.25 |
| | .2 | 2 |
| | .1 | 1 |
| | .05 | .5 |
| | .02 | 2 |
| | .01 | 1 |
| | .005 | .5 |
| | .001 | 1 |
| | 0 | 0 |

$$1 \mu\text{l} \rightarrow 20 \mu\text{l} = 0.1 \text{ U} / \mu\text{l}$$

$$> 10.01 \text{ U} \leftarrow 1/10$$

$$1/10$$

200 μ l DNTP
4 μ l primer
50 μ g Template
2 mM Mg

94° 30"
(94° 30") 85°
68° 5'

| | |
|------------------|------------------------|
| H ₂ O | 1120 |
| x buffer | 140 |
| Tag | 28 |
| DNTP | 28 |
| mixture 1 | 10.5 |
| 2 | 11.8 |
| Template | 56.0 (50 μ g / Rx) |
| Tag | 5.6 |
| | 1400 |

has to be repeated again
low into bottom.
pink Rxs too.

[Signature]

Prepared & Understood by m ,

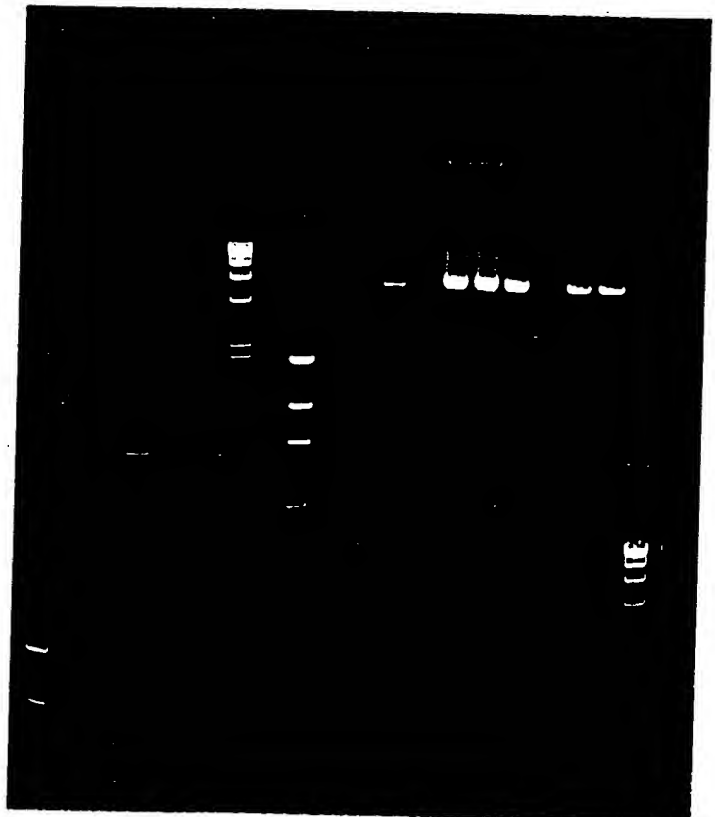
Date

Inventor

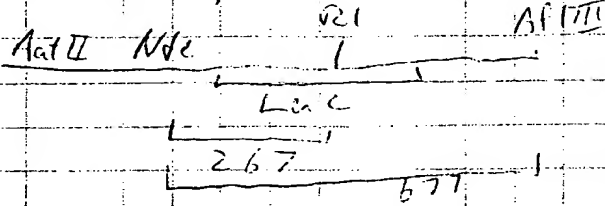
Record

J. Subraman

12/22/94

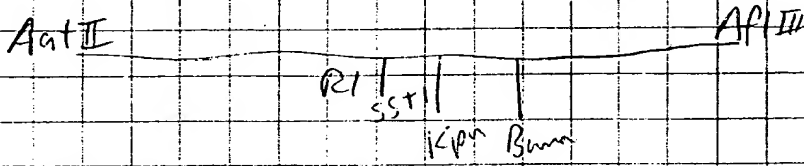


age N — cut on at Nde I to see if all of locus is present even though Aat II is missing



Nde I is OK in buffer 4 (NEB) (P169)
 expect 677 bp Nde I / Afl III band

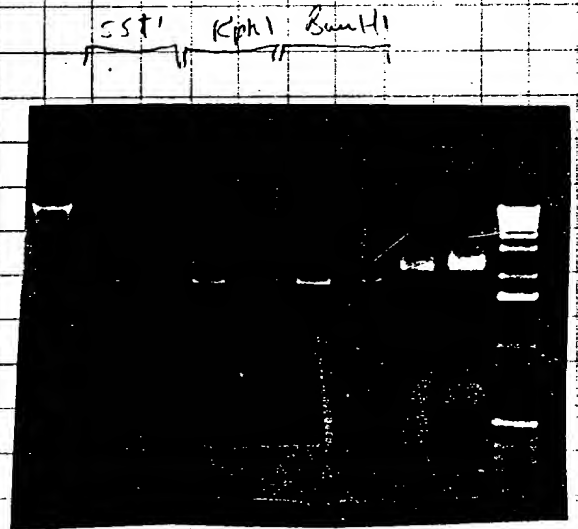
cut with Sst I, Kpn I or Bam HI to see if missing, R1 site is only a point mutation, (all OK in NEB buffer 4)



(for cloning #20
 (tubes 2, 4, 6 below)
 see if Sst I, Kpn I or Bam
 can cut in MCS

tube # 1 2 3 4 5 6 7 8

| | | | | | |
|----------------|-------|---|---|---|---|
| A #1 | 5 | 5 | 5 | 5 | ✓ |
| (no cloning) | | | | | |
| missing R1 | 5 | 5 | 5 | | ✓ |
| missing Aat II | | | | 5 | ✓ |
| + II | 0.1 | → | → | → | ✓ |
| I III | 0.3 | → | → | → | ✓ |
| I | 1 | 1 | | | |
| I | | 1 | 1 | | |
| HI | | | | 1 | 1 |
| 21 | | | | 1 | 1 |
| 20 | 11.6 | → | → | → | ✓ |
| buffer 4 | 2 | → | → | → | ✓ |
| mult. | 20 µl | | | | |

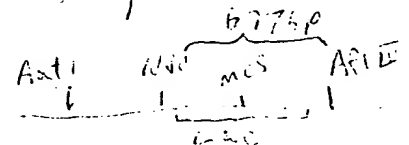


37°C 2 hr

G A A T T C G A G C T C
 R1 Sst I

point mutation

Sst I cuts but R1 did not (P106)
 is mutation is no more than 1 bp downstream
 from the R1 site and may even be a



To Page No. _____

We may give the 677 bp fragment expected if multiple bands are seen

sed & Understood by me,

Erin Baker

Date

2/16/95

Invented by

Recorded by

Date

1/13/95

Project No. _____

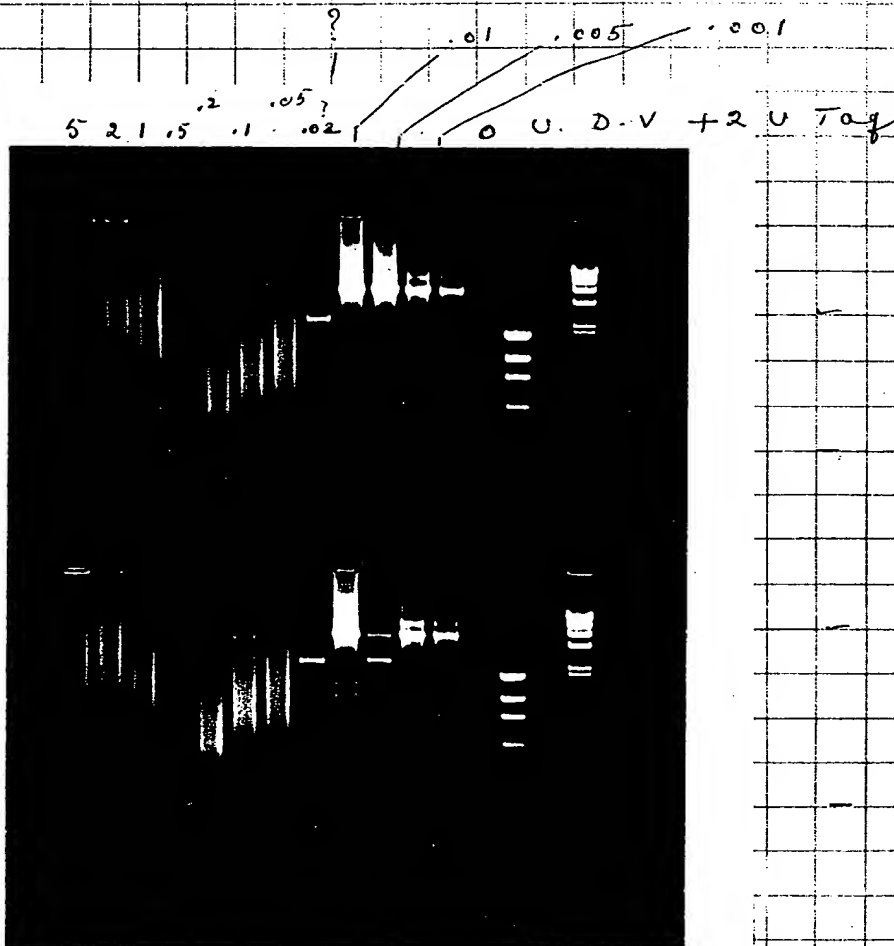
144 12/27/94

Book No. _____

TITLE _____

From Page No. _____

The gal. was run.



Result:

Tag 20 / 50 pg & yield

mispriming still under these conditions

Higher conc of Dap in the presence of Tag no product

0.02 U + 2 U Tag no product either

but with 0.01 U + 20g Tag plenty of product

Is this Dap vent too, or mistake in diluting??

check again w. new Rx.

To Page

Witnessed & Understood by me,

Date

Invented by

Date

Record d by

K. Blahman

12/28/94

34P 733

Project No. _____
Book No. _____

109

Age N — Same as P 99
make 40 μ l of 32P 733

| | | |
|----------------------|---------------------|---------------|
| | ① (dT) | ② (dU) |
| | 20 μ l | 20 μ l |
| 2863 | | |
| or #677 (dT) | 5.05 μ l | ✓ |
| 10 pmol/ μ l | | |
| #678 (dU) | | 7.5 μ l ✓ |
| 6.76 pmol/ μ l | | |
| N Tso pH 7.5 | 2 | 2 ✓ |
| H ₂ O | 40 | 56.5 ✓ |
| | <u>42</u> | |
| | V _A = 66 | |
| 5', 70°C → cool slow | | |

To Page No. _____

Designed & Understood by me,

Date

Invented by

Date

Charles A. Boland

2/16/95

Recorded by

176-95

Page No. _____

repeat: PCR amplification with 20g enzyme +
different amount of Deep Vent.

Repeat of previous expt, 4 of points below.

200 μ M dNTP

D.V.:

0.4 μ M primers

50 pg Template

2 mM Mg

2 U Tag

1, 0.5, 0.2, 0.1, 0.05, 0.02, 0.01,
0.005, 0.002, 0.001, 0

0.1 μ l diluted to 0.1 μ l \rightarrow $\frac{1}{10} = 0.01 \mu$ l \rightarrow $\frac{1}{10} = 0.001 \mu$ l
in 1x buffer w/o Mg.

prepared premix 25x, done in duplicate.

45 μ l of " + 5 μ l of different amount of enzyme.

H₂O10x buffer 125 μ l

dNTP 10 mM 25

Mg 100 mM 25

primer 1 10.6

2 9.5

Template 25.0

112.5 \leftarrow added 2.5 μ l Tag = 250

removed 40 μ l = w/o any enzyme

After adding Tag, mixed & aliquoted 45 μ l / rx to diff. tubes

added Deep Vent diluted different conc.

To Page N _____

Seen & Understood by m ,

Date

1/9/95

Invented by

Date

Recorded by

K. S. S. S. S.

12/27/94

11, 20

| | | | | | | | | |
|-----------|----|----|----|---|---|----|----|----|
| 207 Tng + | 0 | 1 | 5 | 2 | 1 | 05 | 02 | 08 |
| 0 | 15 | 12 | 11 | 9 | 7 | 5 | ✓ | |

20 Tagt 3 17 19 21
101 105 102 100 108 DV

Recorded by _____

12/28/94

Project No. _____

Book No. _____

TITLE _____

110

From Page No. _____

① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩ ⑪ ⑫

32P 733 .2863
(JT)

5 —————→

32P 733 .678
(JT)

5 —————→

10 x Vent buffer
4 JNTPA 10 mM each
4 CTG-TP 2.5 mM each

5 μ l

1 μ l

44

rTag 3 μ l EKBT1
dilute to 0.25 units μ l

→ 2 2 2 2 2 — 2 2 2 2 —

* Vent DNA polymerase

0.125 μ l

1

1

0.5 μ l

1

1

2 μ l

1

1

1

1

1

H₂O

34 35 37

—————→

39

37

—————→

39

50 μ l

70°C, remove 10 μ l to 5 μ l stop at 2, 5, 10 min

pol mix

rTag 3 μ l

2

20.67

1.85

0.5

Vent 2 μ l

*

1

2

3

*

Top storage buffer

22

44.3

20.67

5.1

✓

1/2 27

48

24

9

Add

2 1

3

3

3

1

note ix vent buffer is 2 mM MgSO₄

* dilute with Vent dil storage buffer

To Page N

Witness d & Understood by me,

Date

Invented by

Date

Deanne Bolamp

2/16/95

Record d by

1-17-95

Tag No. _____

purpose: To check again pMC9 w/ Deep vent } alone
Tag

50 μ g Template
200 μ M dNTP
1.4 μ M primer
2 mM Mg

prepared premix 45 μ l / rx
added diluted enzyme in 5 μ l

for DV tried: .5 u.

.2

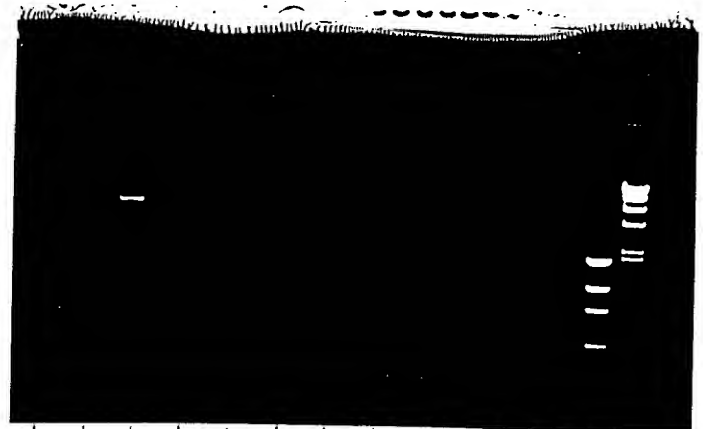
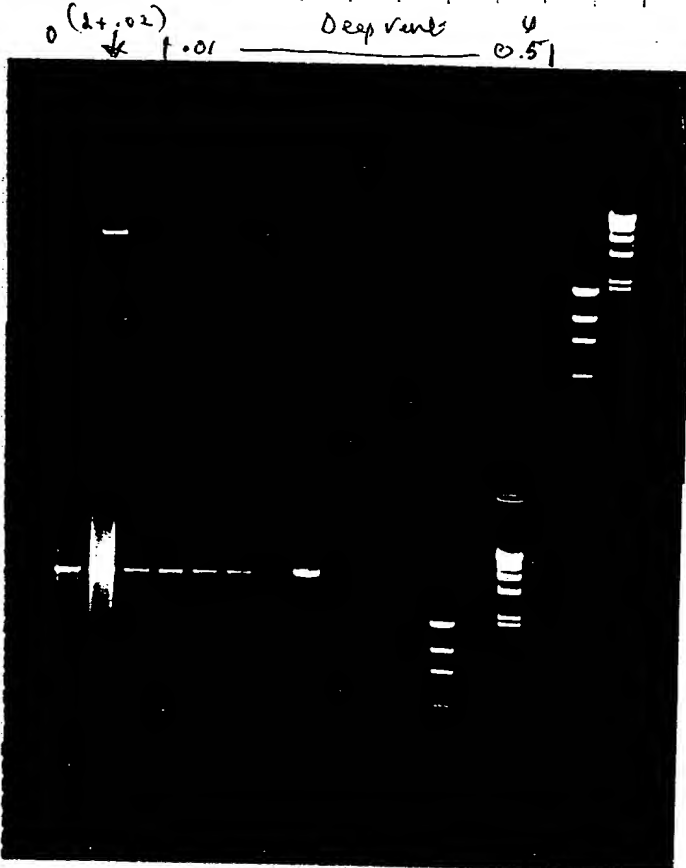
.1

.05

.01

for Tag: 5, 2, 1.5, 0

Mix: (2+.02) and (1+.01)



4 5 2 1.5 0 (1+.01)
(2+.02) mix

Increasing less than what
con of Tag one would expect
increased product
yield

↑ order?

To Page No. _____

Used & Understood by me,

Date

Invented by

Date

Recorded by

12/28/94

K. Staraman

SDMTP sequencing reactions

Project No. _____

Book No. _____

Exhibit 30
Appl. No. 09/558,421

111

| Tube N | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 55 |
|----------|----|----|----|----|----|----|----|----|----------|
| | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | |
| | 1 | | | | | | | | ✓ |
| buffer 1 | | | | | 1 | | | | ✓ primer |
| mix | 2 | | | | 2 | | | | ✓ |
| | | 2 | | | | 2 | | | ✓ |
| | | | 2 | | | | 2 | | ✓ |
| | | | | 2 | | | | 2 | ✓ |
| 5.5 | | | | | | | | | ✓ |
| 4.5 | | | | | | | | | ✓ |
| 0.5 | | | | | | | | | ✓ |
| 10 µl | | | | | | | | | ✓ |

5 min, 70°C, → add 5 µl stop

Sequencing see P 27, 4

| | | | |
|-------------------------------|------|------|---|
| ³² P 733-2863 (dT) | 12 | | ✓ |
| P 733-677 (dA) | | 12 | ✓ |
| DTT 0.1M | 1 | 1 | |
| sequencing kit buffer | 2.27 | 2.27 | ✓ |
| | 2.23 | 2.27 | ✓ |
| | 17.5 | 17.5 | |
| 3.5 | ✓ | ✓ | ✓ |
| 2.5 | ✓ | ✓ | ✓ |
| 2.5 µl | | | |
| Sequencing | 0.2 | | |

ACGT ACGT

To Page No. _____

Read & Understood by me,

Ernesta Polanco

Date

2/16/95

Invented by

Recorded by

Date- 17.55

1-18-95

TITLE

Purpose:

Hg titration in PCE w/ pMCP with Tag
and Tag + D.V.

did at 200 mg d nTP.

14 May summer
30 May limy slate

Mg : 1, 1.5, 2, 2.5, 3 mM

$$(T_1 - T_3) \quad 2N$$

(2 ± 0.02) numbered

prepared premix with Ta_2O_5 or $\text{Ta}_2\text{O}_5 + \text{O}_2$ respectively 4.57

added dif amount of Mg in sol

same cycling conditions

Result :

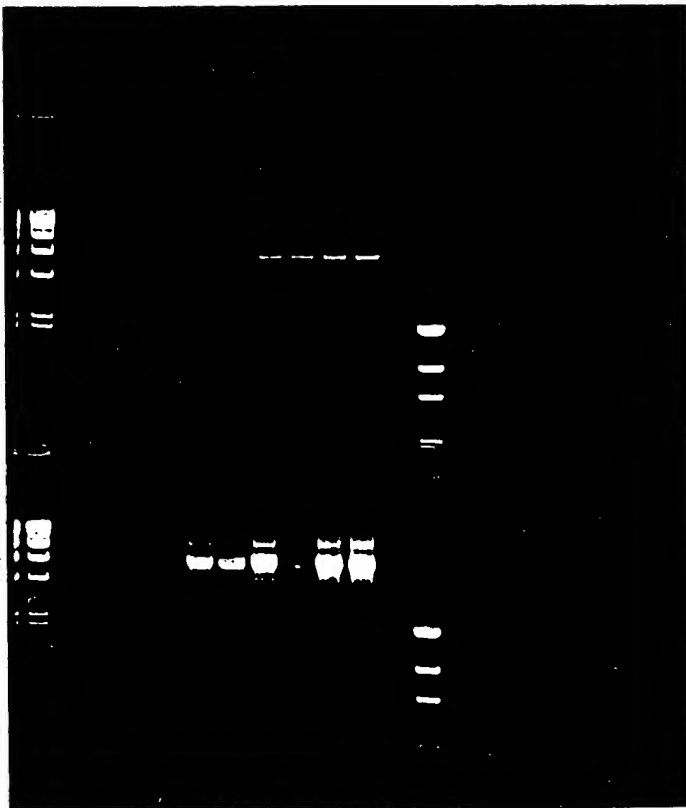
Tag 20.

20 g Tag : increase
the chem of Mg for 2.5
3

2 V Considerably since
the products yield

2u. $T_{eq} = 0.02 \text{ u}$ DV
 even at 1.5 mV Mg
 product seen.

more product as
as more misprints
with increasing con
ng.



1 1.5 2 2.5 3 mV Tag \pm DV
2 \pm .02

12/28/84

rk. Abraham

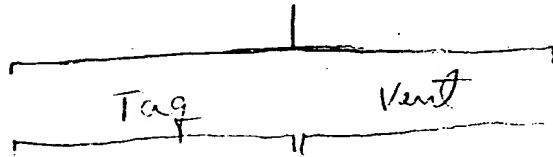
DU.GEL

- 01/19/95 - 09:20 pm

2.00x Counts

29.99 ☐ 200.

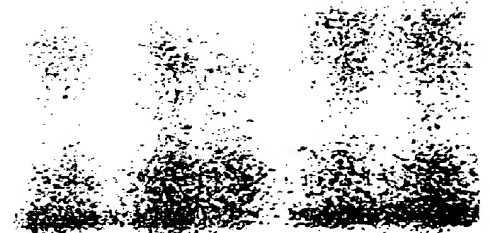
- dATP and (T) primer



min 0 2 5 10 0 2 5 10

U -
 A -
 A -
 U -
 U -
 pri

← post incorp block
 ← pre incorp block



← primer degraded

| | | | | | | | | | | | |
|-------------------------------|--|--|--|--|------|--|-------------|--|--|------|---|
| | | | | | | | | | | T | P |
| Witnessed & Understood by me, | | | | | Date | | Invented by | | | Date | |
| | | | | | | | Recorded by | | | | |

Page No. _____

upset: To check 1. Tag def amount (since 1.50 works in one case)
 2. mix of (Tag + DV)
 against

3. freshly made (added separately)

| Tube | U of Tag | # Tube | (T + DV) |
|------|-------------|--------|-------------------|
| 2 | 5) left out | 13 14 | .5 ml .50 + .0050 |
| 4 | 2.5 | 15 16 | 1 1 + .01 |
| 6 | 2 | 17 18 | 1.5 1.5 + .015 |
| 8 | 1.5 | 19 20 | 2 2 + .02 |
| 10 | 1 | 21 22 | 5 5 + .05 |
| 11 | 0 | | |
| | ↑ | | |

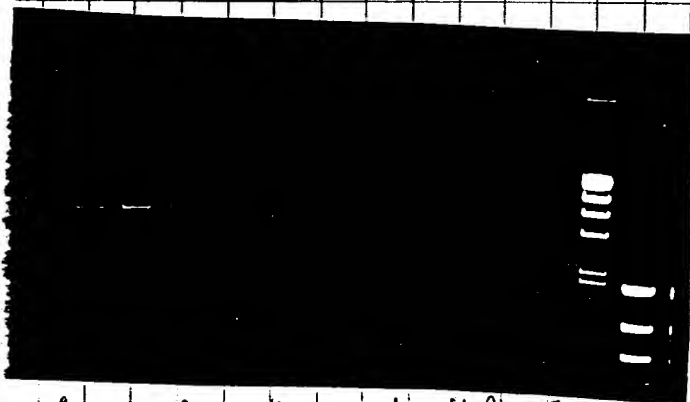
(added in 5ml +) 45ml rx (+ added in 5ml)

under cycling conditions.

10 Tag + D.V. .005, .01, .020 - diluted in 5ml.
 20 Tag + D.V. " " "

Result: w. Tag above
 10 barely results in
 product.

- frequent freeze-thawing?
 (



To Page No. _____

ed & Und rst od by m ,

Date

Inv nt d by

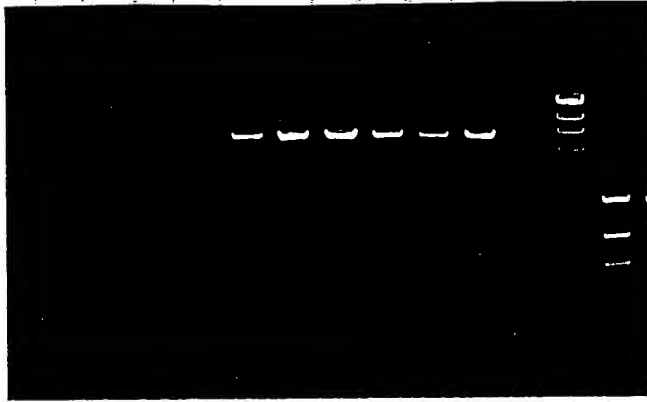
Date

Recorded by

J. Silvarman

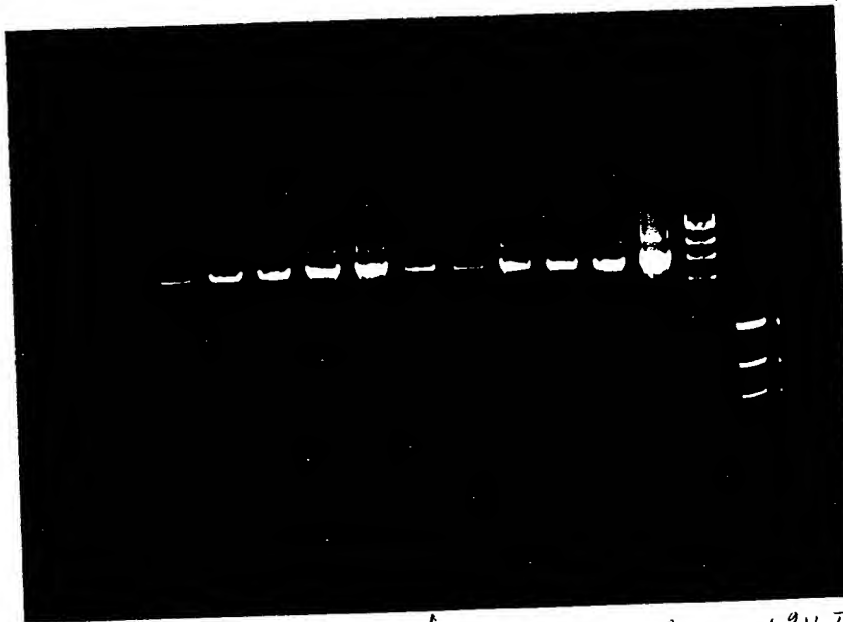
1/3/95

premix:



| | | | | | |
|--------------------------|-----------------|--------------------|-----------------|-----------------|-----------------|
| $\frac{0.5}{0.005}$ | $\frac{1}{.01}$ | $\frac{1.5}{.015}$ | $\frac{2}{.02}$ | $\frac{5}{.05}$ | $\frac{10}{.1}$ |
| | | | | ml | ml |
| ✓ repeated again - books | | | | | |

fresh mix



10 Tg + .005 .01 .020 | .005 .01 .02 + 20 Tg

2x² - 1x - 6

per

CU

- Ruth with 10
level of fog
increasing the
amount of D
from 0.005
to 0.2
the product
goes up!

man's seen
he holding

Date 1/4/95

Recorded by _____

typed by
K. S. Kneaman

7/5/25

To Page 1

Project No. _____

Book No. _____ TITLE _____

Miniprep for Ayoabs
PCR

114

From Pag No. _____

miniprep #

Ayoabs PCR conditions

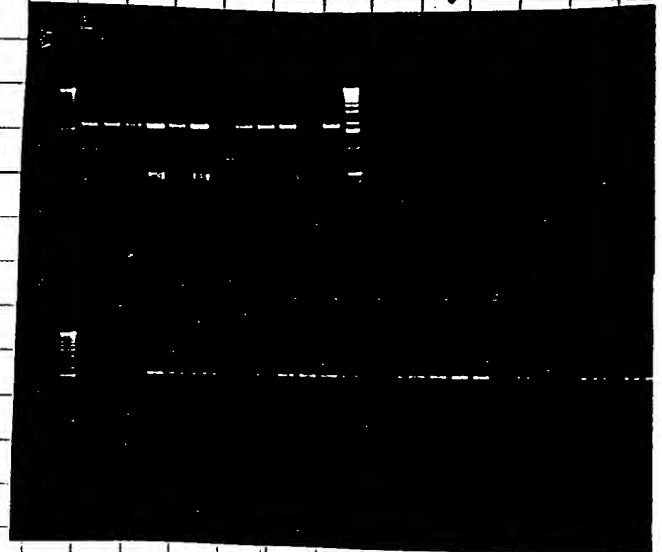
| Tag | Tag + Deep Vial | W/N |
|---------|-----------------|-----|
| # 1-20 | + | 0 |
| 21-40 | + | .05 |
| 41-60 | + | 0.1 |
| 61-80 | + | 0 |
| 81-100 | + | .05 |
| 101-120 | + | 0.1 |
| 121 | Blue colony | |

grow O/N 30°C, 2 ml circle grow + 100 µg/ml Amp

miniprep same as p41, 4 using 1 ml cells

digest as per P 93 A1F II, Aat II, Eco RI 5 µl miniprep
1-40 on 42 well comb, load 10 µl
conclude resolution not good
enough for ~500 bp range

1-40 on 30 tooth comb, load
need more DNA in digest and load 2



10

20

To Pag N

Witnessed & Understood by me,

Deanna Polanco

Date

2/16/95

Invented by

Recorded by

Date

127-55
30-55
12A

Repeat digest of P114 for fidelity
assay: use 10 μ l miniprep

Project No. _____

Exhibit 33
Appl. No. 09/558,421

Book No. _____

115

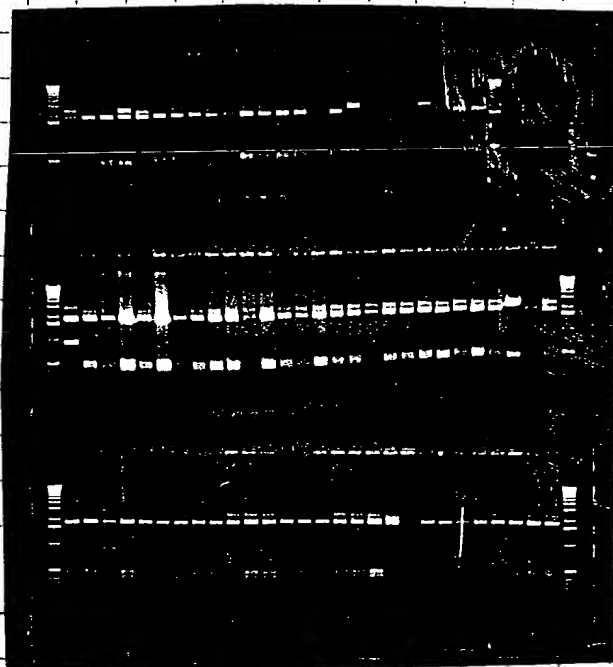
ag N —

| | | |
|------------------|-----------|---|
| NEB buffer | 2 μ l | ✓ |
| 1/1 Afl III | 0.5 | |
| 1/1 Aat II | 0.1 | |
| 1/1 Eco RI | 0.5 | ✓ |
| H ₂ O | 7.1 | ✓ |

VP = 10 μ l

digest 10 μ l miniprep
VP = 20 μ l

digest 2 hr 37°C load 20 μ l

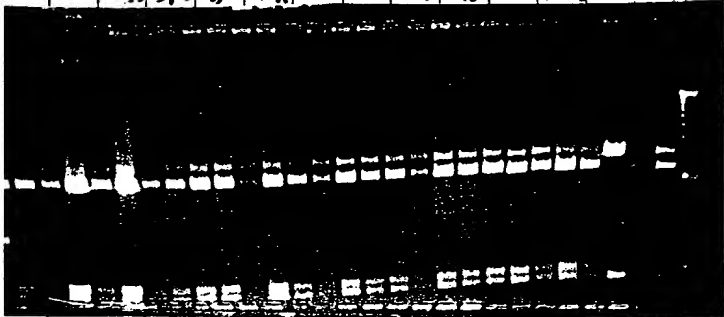


miniprep
← 57-81

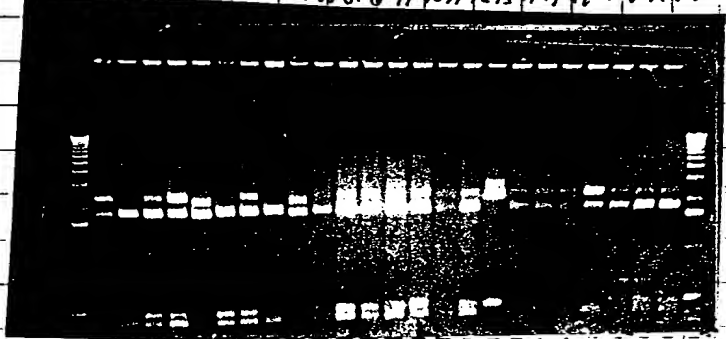
← 29-56

← 1-28

0.5 mm Mn 1 0.1 mm Mn

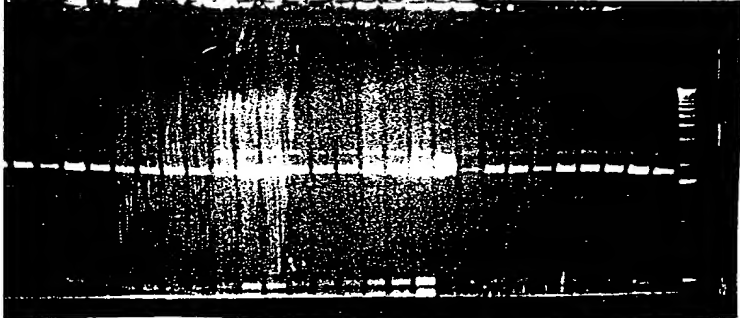


no Mn, + Vent



47
= 47

no Mn 0.5 mm Mn



↑ Oel ↑ ? ↑ 47

use full length
for #13
rel P123

To Page No. _____

sed & Understood by me, .

Date

2/16/95

Invented by

Recorded by

Date

1-30-95

Project No. _____

Book No. _____

TITLE _____

152

1/3/95

From Pag No. _____

Applications:

Amplification from plasmids:

Purpose: - To start the cultures of different size fragments in pDELTA 1

- all glycerol stocks obtained from this young

| | | | |
|--------|-----|----|---------------------------|
| 6.4 Kb | PYA | 21 | 3 ml of LB + 100 µg/ml |
| 8.0 | | 57 | freshly made Amp |
| 10.5 | | 20 | & stock of glycerol stock |
| 20.0 | | 47 | overnight at 37° |
| 29.0 | | 17 | |

1/4/95

- except for # PYA 20 - 10.5 Kb rest of them grew quite well

It was regrown again overnight with fresh stock.

- Rest of them 1.5 ml of each was miniprep. using alkaline lysis method. see page 139. stored at 4° suspended in 25 µl of 1X TE.

- Each culture was diluted 10 + 990 (100) - 1/100 dilution plated 25 µl of diluted culture onto per made Amp plates, incubated at 37°, overnight

6.4 & 8.0 57 and 21 were overgrown but good isolated colonies in the periphery of the plate.

21 & 17 gave just 4 colonies in 2 plates!

20 & 47 quite a few with spread, full blown large colonies

- Replated further diluted # 57 & 21 for further use.

To Pag

Witness & Understood by m ,

Dat

Inv nt d by

Dat

Recorded by

R. Stharaman

1/5/95

Project No. _____

Book No. _____

Results P 115

116

From Page No. _____

#1-20, Tag 0 mm

17 19

(no Ant II) (no Ant III)

1

X

21-40, Tag 0.05 mm Mn

17 20

X

41-60, Tag 0.1 mm Mn

18

2

61-80, Tag 0 mm
+ Deep Vent

12 17

X

1

2

X

81-100, Tag 0.05 mm Mn
+ Deep Vent

18

2

101-120, Tag 0.1 mm Mn
+ Deep Vent

16

4

See well

Table on P 124

after Ant II and SST
cuts

* 900mer lacks R1 site in mcs

* no result, i.e. not enough DNA
to be sure about cut.

0.1 mm Mn, Tag + Deep Vent

105

120

confirmed deletions
miniprep #19, 61, 65only 410 or 465 removed
so to 2.2 bp1.8 bp
has 410 and 465 removed

miniprep #



Witnessed & Understood by me,

Deena Polansky

Date

2/16/95

Invented by

Recorded by

Date

1-31-95

P116 continued
Experiment done on P. 123

Project N. _____
Book N. _____

117

ag No. _____

Still Needed 8

cut with Orf I to see if full length lac Z is present
(assuming either Afl III or Aat II recognition region
had a point mutation/generation). Therefore the "410" and "465" bp

miniprep # 54, 58, 64, 73, 87, 98, 103, 108, 113, 120

plus Aat II, Afl III

cut with Sst I to see if R1 site in MCS was
a point mutation (or very small deletion
(all on P107 at bottom) resulting in the "900mers"

miniprep # 3, 29

Recut with 17 μ l miniprep and load 30 μ l?
2.5 μ l reaction

to try to resolve the "No results"

miniprep # 20, 39, 71, 74, 75, 76

To Page No. _____

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_____ in a Bolam

Date

2/16/95

Invented by

Recorded by

Date

1-31-95

Project No. _____

Book No. _____

TITLE *Work at Frederick*

From Page No. _____

| | CPM | TIME |
|--------------|----------|------|
| A1 { 1 | 4976.00 | 0.50 |
| A1 { 2 | 5216.00 | 0.50 |
| A1 { 3 | 4500.00 | 0.50 |
| A1 { 4 | 16920.00 | 0.50 |
| A0 { 5 | 17020.00 | 0.50 |
| A0 { 6 | 16156.00 | 0.50 |
| A1 { 7 | 3926.00 | 0.50 |
| A1 { 8 | 3822.00 | 0.50 |
| A1 { 9 | 4034.00 | 0.50 |
| A2 { 10 | 15974.00 | 0.50 |
| A2 { 11 | 16520.00 | 0.50 |
| A2 { 12 | 15478.00 | 0.50 |
| A3 { 13 | 4684.00 | 0.50 |
| A3 { 14 | 4752.00 | 0.50 |
| A3 { 15 | 4606.00 | 0.50 |
| A0 { 16 | 17622.00 | 0.50 |
| A0 { 17 | 16806.00 | 0.50 |
| A0 { 18 | 17742.00 | 0.50 |
| LT1 { 19 | 4186.00 | 0.50 |
| LT1 { 20 | 3966.00 | 0.50 |
| LT1 { 21 | 3986.00 | 0.50 |
| LT2 { 22 | 14842.00 | 0.50 |
| LT2 { 23 | 14704.00 | 0.50 |
| LT2 { 24 | 15620.00 | 0.50 |
| L { 25 | 4458.00 | 0.50 |
| L { 26 | 4644.00 | 0.50 |
| L { 27 | 3970.00 | 0.50 |
| L { 28 | 16730.00 | 0.50 |
| L { 29 | 16914.00 | 0.50 |
| L { 30 | 15684.00 | 0.50 |
| L3 { 31 | 4864.00 | 0.50 |
| L3 { 32 | 5020.00 | 0.50 |
| L3 { 33 | 4538.00 | 0.50 |
| L3 { 34 | 15236.00 | 0.50 |
| L3 { 35 | 17922.00 | 0.50 |
| L3 { 36 | 17898.00 | 0.50 |
| Aguasol { 37 | 12.00 | 0.50 |
| Aguasol { 38 | 16.00 | 0.50 |
| Aguasol { 39 | 16.00 | 0.50 |

delivered 10µl with p10 (wiped tip)
rinse 3x into 4 ml aguasol

each dilution had 3µl of 1µCi/ml ³H TTP

To Page 1

Witnessed & Understood by me,

Date

Invented by

Date

Deanna Polansky

2/16/95

Recorded by

1/25/95

New rTag dilutions

g N _____

#

EKB T1

77.4

18.6 μ l323 units/ μ l (P91)

Tag dilution buffer

4922.6 μ l1981.4 μ l $V_f = 5$ ml
(5 units/ μ l) $V_f = 2$ ml
(3 units/ μ l)

both are labelled "1-31-95 rTag"

To Page No. _____

Read & Understood by me,

Date

Investigated by

Date

Deena Solari

2/16/95

Recorded by

1-31-95

6.4 kb

Page No. _____

purpose: To amplify 6.4 kb and 8.0 kb from plasmid

used F + R (non do) primers

50 μ l rx. 200 μ l dH₂O each cycling: 94°; 1'
 .4 μ l primers
 2 mM Mg used buffer B
 Template ?
 1 μ l enzyme pre mixed (1:0.01) $\left(\begin{array}{l} 94^{\circ} \text{ } 30'' \\ 60^{\circ} \text{ } 45'' \\ 72^{\circ} \text{ } 3'' \end{array} \right) \times 25$

prepared enough premix for 20 rx:

6.4 kb:

all done in duplicate.

included purified }
 prep at a known }
 concentration }
 used 50 μ g + 100 μ g
 (Tag 50) just one.

mini prepped, unknown }
 concentration (from }
 the amount of colonies }
 in 1/100 dilution) }
 conc. should be quite }
 high in the mini prep. }
 diluted to 50 μ l }
 used .5 μ l and 1 μ l

plasmid - picked a single isolated colony directly
 into the reaction mix containing all the rest of the stuff
 done in duplicate

8.0

no purified stuff available

mini prep - }
 unknown conc }
 lot of colonies }
 from 1/100 \rightarrow 25 μ l }
 dilution }
 .5 and 1 μ l
 (out of 60 μ l from
 1.5 ml culture)
 plasmid - 2, one in each - done in duplicate

T Page No. _____

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Dat

1/9/95

Inv nt d by

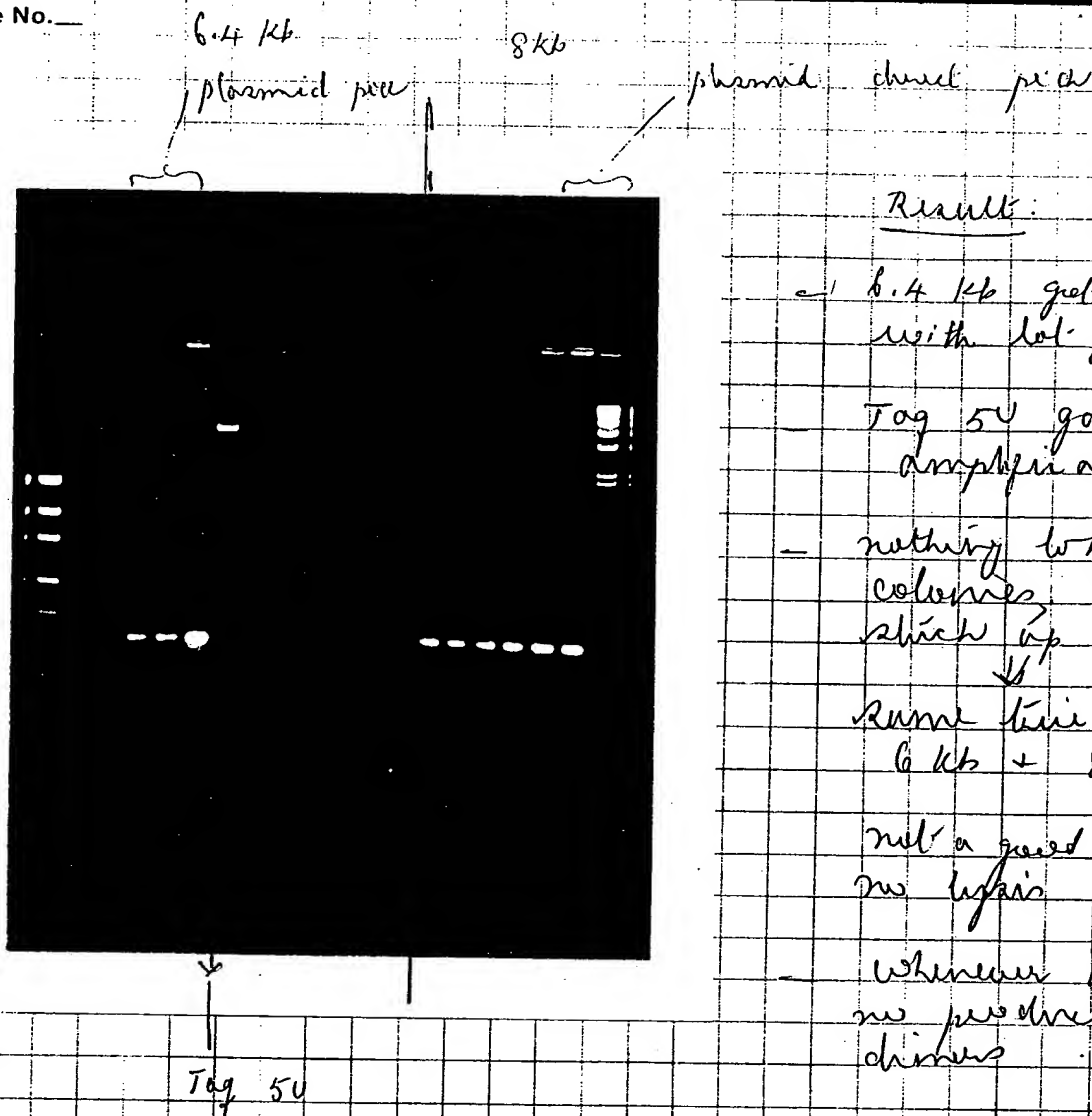
Recorded by

J. Sitarawan

Dat

1/5/95

From Page No. _____

Results:

- 6.4 kb got amplified with lot of mispriming

Tag 50 gave good amplification

- nothing to be seen for colonies, lot of stuff which up in the air

Same time with both 6 kb + 8 kb

not a good way w/ no lysis at all,

- whenever there was no product lots of products

- amount of primers to be found enough.

* check alternate cycling conditions to get rid of mis priming

* lysis in PK and just water has been checked next

* make 6.4 kb to work first

T Page No

With ssed & Und rstood by me,

Dat

Inv nted by

Dat

R corded by

Dr. Subramaniam

1/9/95

Project No. _____

Book No. _____

TITLE

Accuracy of delivering 1 μ l
with P2 pipetman for Tag Storage

From Page No. _____

add 1 μ l, 10 times to a weigh boat with
a drop of H₂O in it so tips can be rinsed
several times. Use storage buffer at 0°C (on ice)

add H₂O Tare 0.00001 μ l

2

3

4

5

6

7

8

9

10

0.0119

 $(\frac{94}{100}) =$

0.011

note 10 μ l SB = 0.01
.00943.0 \sim 1.1 μ l was added instead of the 1 μ l intendedconclude ~~2 μ l~~ 1 μ l is OK to add to unit assayconclude 2 μ l is better to add for units

stock for Tag unit assay

use

CP in unit assay

for 6.6 & 7 Rxs

3 ml

0.5 M TAPS pH 7.3

150 μ l25 mM \rightarrow

✓ X 206

120 μ l1 M MgCl₂6 μ l

2 mM ✓

0.1 M OTT ✓ 3

1 ml

3 M KCl

50 μ l

50 mM ✓

10 mM DKT ✓ 6

4.12 ml

VP = 206

✓ \rightarrow 10 mM DKT ✓ 6use 137.5 μ lfor 2 μ l unit assayuse 230 μ l / 2 μ l Tag unit assayH₂O ✓ X 2.293

CP = 3

use 137.5 μ l / 50 μ l 4 ml

To Page No.

Witnessed & Understood by me,

DeeAnna Polamp

Date

2/16/95

Invented by

Recorded by

Date

2-1-95

6.4 kb

ag No. _____

Purpose: To repeat & optimize preliminarily 6.4 kb.

Tried mini prep DAPI as a control

Will try 2 dif. cycling conditions 3 step as well as 2 step.

Colonies will be lysed in 2 different ways 1. in PK (single)
2. in H₂O colony
unlyzed will also be included again. bufferconditions: - since 200 µM DAPI + 2 mM pH 7.5, 10 mM Tris-HCl
4 µM puerh has Mg 1 mM EDTA
worked with Tag 5U, the same 50 µg/ml PK
conditions will be used. ↓used 2 µl of mini prep - can unknown (used DAPI (3).
still have to run gel)Tried dif. enzyme conc 1, 2, 5 and 1:0.01, 2:0.02, 5:0.05
Tag Tag & DVColony lysis: Since these colonies were so minute after or
at 37° pooled 5 or 6 colonies in a single area -
spotted 2 µl of lysis buffer or H₂O mixed & pipetted out the
liquid on to a tube containing 10 µl of lysis buffer
or H₂O

Colonies in PK lysis 55°, 15' → 95°, 15'

in H₂O 95°, 15'(Added ~ 5 µl of H₂O) pooled all three tubes together
and made up the volume to 50 µlShould have picked more for more reactions
used 10 µl / Rx - appropriately either PK lysed or H₂O lysed or
colony itself 10 µl H₂O To Page N

Used & Understood by me,

Date

Invented by

Date

Recorded by

K. Subraman

1/6/95

From Page No. _____

for mini-prep DNA : prepared premix with template
for colony : added them later

mini-prep premix : 25x

| | | |
|------------------|------------|-----------------------------|
| A | | |
| dNTP | 25 μ l | (200 μ M each / Rx) |
| P.P | 5 | (250 μ M) 0.4 μ M |
| R.P | 5 | 0.4 μ M |
| Template | 25x2 | last exp. used 1.5 + 1 / Rx |
| mini-prep | | = 2 μ l / Rx |
| H ₂ O | 415 | |
| 500 | | 200 μ l / Rx |

premix B: 5x

| | | Tag | | | Tag + DV | | |
|------------------|------|-----------------|------|----|----------|------|------|
| | | 1 | 2 | 5 | 1:01 | 2:02 | 5:00 |
| (2mM) Buffer B | 50 | | | | | | |
| 100x enzyme | 5 | 1 | 2.5 | 5 | 10 | 25 | |
| H ₂ O | 99.5 | 99 | 97.5 | 95 | 90 | 75 | |
| 150 | | | | | | | |
| | | 30 μ l / Rx | | | | | |

step 2
3 cycle

94°, 3'
20 (94°, 45"
55°, 30"
72°, 3')

2 step 1

94°, 3'
94°, 45"
68°, 5'

T Page 1

Witnessed & Understood by m ,

Date

Invented by

Date

Recorded by

Dr. S. S. S. S.

1/6/94

| age No. _____ | | 2 step | 3 step |
|---------------|--|---------|--------|
| Tube # | | | |
| 1 | | 1 | 13 |
| 2 | | 1 | 14 |
| 3 | | 2 | 15 |
| 4 | | 2 | 16 |
| 5 | | 5 | 17 |
| 6 | | 5 | 18 |
| 7 | | 1 : .01 | 19 |
| 8 | | 1 : .01 | 20 |
| 9 | | 2 : .02 | 21 |
| 10 | | 2 : .02 | 22 |
| 11 | | 5 : .05 | 23 |
| 12 | | 5 : .05 | 24 |

Colonies

Pre mix A : 15 x

Mix B : 5 x as earlier :
for T + D.V

dntp 15

primer 3

" 3

- 150 (template 10 µl / Rx (the added later))

L20 129

150 → 10 µl f Rx

20 µl → + ←

added 30 µl / Rx
appropriately either
Tag alone or Tag + Rx

changing condition same as mini prep.

for 3 step cycle, 2 step not done. " didn't
have much template left from
lysed plasma

| | |
|----|---------|
| 25 | 1 + .01 |
| 26 | 2 + .02 |
| 27 | 5 + .05 |

PK lysed

.35, .36 (20 Tag)

Hw plain.

28 } 11,20

29 } lysed

30 }

31 }

32 }

33 }

straight piece

is d & Understood by m ,

Date

Inv nt d by

Dat

T Pag No. _____



1/6/95

Rec rd by

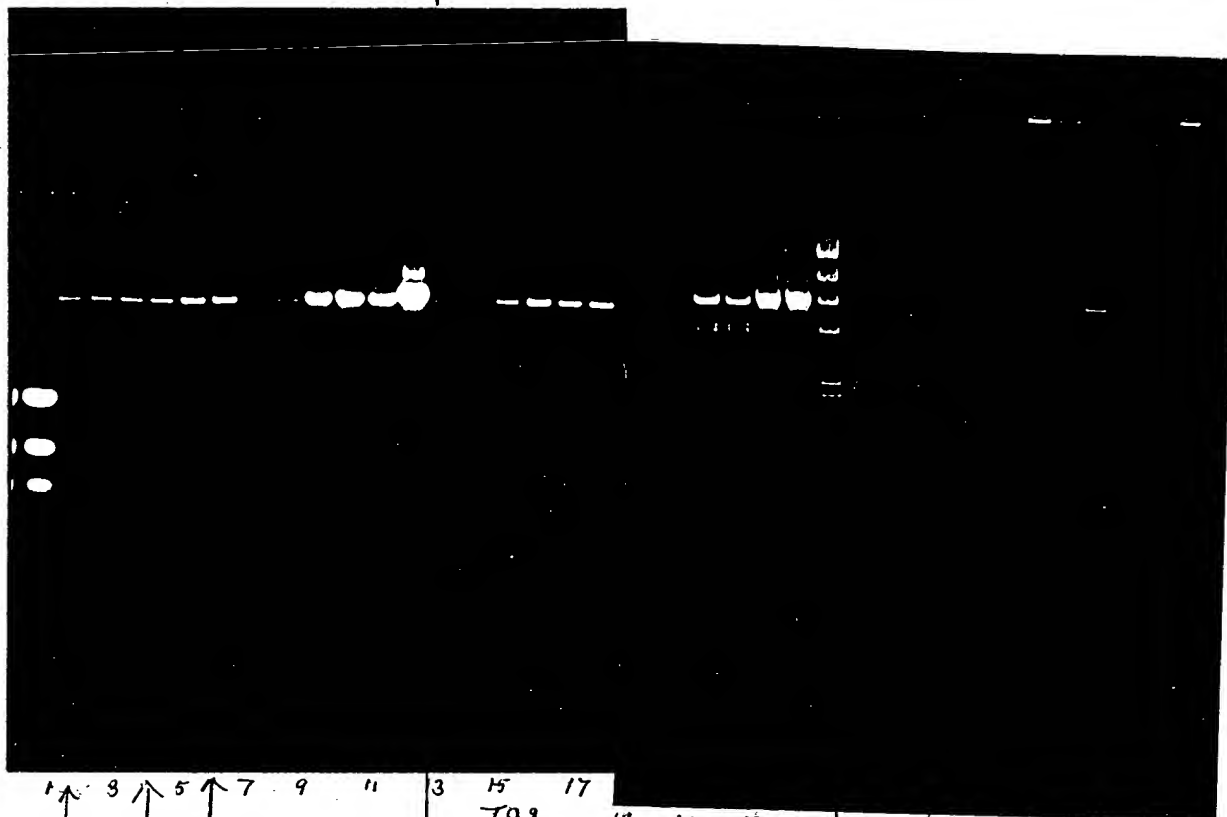
S. Sitarman

1/6/94

From Page No. _____

2 step
cycle

3 step cycle



1 3 5 7 9 11 13 15 17
Tag

19 21 23
T + DV

PK direct
H2O pack

Unit 1
enzyme
Tag + DV

1 2 5 1, 3, 5

Enzyme
Mix

PK H2O direct
tagged tagged pack

W Tag + DV

W Tag + DV

mini prep.

Plasmids

Result: Even 3 step gave better product with less mis-
plasmid amp should be done under more control.

To Page 1

Witness d & Understood by me,

Date

1/20/95

Inv nt d by

Recorded by

K. Stareman

Date

1/9/95

* Continued from P 11)

SSI and DSI costs of mutants

Project N ._____

Book No._____

| age N | normal
1 hr | post fast
control | control |
|-----------------|---|--------------------------------------|------------|
| up # | (1) 1 2 3 4 5 6 7 8 9 10 (11) 12 13 (14) 15 16 17 18 19 20 (21) | | |
| buffer 4 | 30 54 58 64 73 87 92 103 118 113 120 30 | 3 29 30 20 39 71 74 75 76 31 | |
| I | 10 10 \rightarrow | 10 \rightarrow 25.6 \rightarrow | |
| I | 2 2 \rightarrow | 2 \rightarrow 3 \rightarrow | |
| I | 1 1 \rightarrow | | |
| IV | | 0.3 \rightarrow 0.45 \rightarrow | |
| II | | 0.1 \rightarrow 0.15 \rightarrow | |
| I | | 0.5 \rightarrow | |
| -R1 | | 0.75 \rightarrow | |
| V | 7 \rightarrow | 0.1 \rightarrow | |
| Vp = 20 μ l | | Vp = 20 μ l | 30 μ l |
| 37°C | 2 hr | | |

was only $\sim 1/2$ wt by 1 hr
at 0.5 wt more ~~long~~

for DndI

| | |
|-----------|-----------|
| mini prep | 6 p |
| # | Proquents |

1 hr

any Dnd I for last 30 min

control

control

control

Dnd I

control

Dnd I

control

12 13 14 15 16 17 18 19 20 21

2 3 4 5 6 7 8 9 10 11

2687

2683

1764

1763

1762

1761

1760

1759

1758

1757

1756

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| | | | | |
|---------|----------------|-----|-----|-----------------------|
| control | 2.7kb (insert) | 1.8 | 0.8 | full length
casp-1 |
| #73 | " | " | " | " |

58,64
87,120

54, 12, 103
113

1.8

Results

full length
cuc-protein

multiprep
 3 and 24
 have full
 length loc
 dures on primers
 of 410, 465 bp
 30 R1 was probably
 small or point
 mutation

all not
results
of P.I. 1
all full
strength
here based
on pressure
of 410.4656
in more
ent here

↑
mine
#75
has no bps
full length
low in
percent

To Page No.____

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Lucia Polanco

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225

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

| <u>miniprep #</u> | <u>Mn (mM)</u> | <u>Deep Vent</u> | <u>Pull length
lac</u> | <u>percent
rearrangements</u> |
|-------------------|----------------|------------------|----------------------------|-----------------------------------|
| 1-20 | 0 | | 19 | 5% |
| 21-40 | .05 | | 20 | 0 |
| 41-60 | 0.1 | | 18 | 10% |
| 61-80 | 0 | + | 17 | 15% |
| 81-100 | .05 | + | 18 | 10% |
| 101-120 | 0.1 | + | 16 | 20% |

To Page 1

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Dat

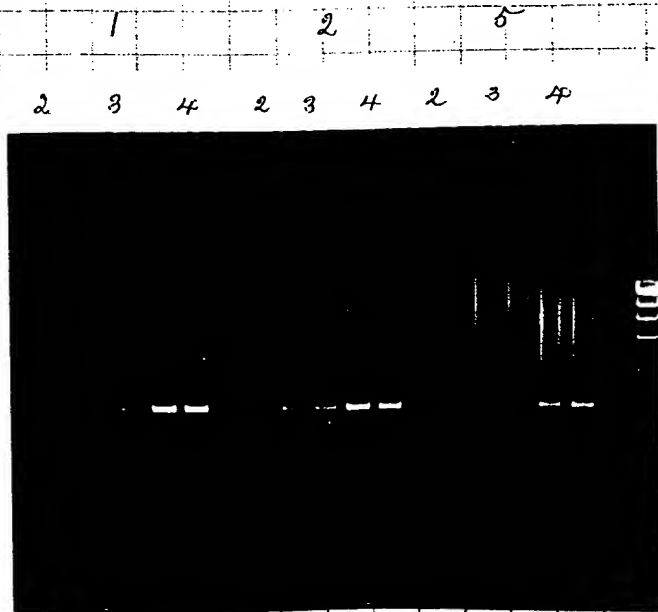
Recorded by

A. Starin

From Page No. _____

| | | | | |
|----|----|---|----|---|
| 1 | 2 | } | 10 | 2 |
| 3 | 4 | | 3 | |
| 5 | 6 | | 4 | |
| 7 | 8 | } | 20 | 2 |
| 9 | 10 | | 3 | |
| 11 | 12 | | 4 | |
| 13 | 14 | } | 50 | 2 |
| 15 | 16 | | 3 | |
| 17 | 18 | | 4 | |

19 Tag 20 2 ml

Result:

cycling has to be optimized - Lot of mispriming
 2 mM Mg didn't work in any of the sets - It
 worked earlier in 25 µl as w Tag.

- amount of template ?

- Increasing the enzyme didn't seem to work.
 as does Mg

- Tag alone at 20 / 50 µl didn't work

- get fresh enzyme.

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Dat

1/25/85

Inv nted by

R corded by

Dr. Subraman.

Date

1/10/84

To Page

Stability study for PCK mix containing 1 unit of Tag

Exhibit 37
Appl. No. 09/558,421

B K N

Assay date is
2-3-95 121

| Age No. | Experiment | Conc | Sub | Reagent | Rxn # | at enzyme added to reaction | Tag with or without A22 | * put 3ul H ₂ O/4 mix to 1X = 48ul mix |
|---------|----------------------------|----------------------|--------|---------|-------|-----------------------------|-------------------------|---|
| 1 | J. Soler of 1-20-95 | | | | | | | |
| 2 | R26 | 0.1% TN (Tween/NP40) | | | 1-3 | 2 (0.05%) | 48ul | 20+2 X Enz gives 1/2 = 50 |
| | | 0.2% BS | Brij | | 4-6 | | | |
| | | 0.2% TX | Triton | | 7-9 | | | |
| | | 0.01% TN | | | 10-12 | (0.0004%) | | |
| | | 0.02% BS | | | 13-15 | | | |
| | | 0.02% TX | | | 16-18 | | | |
| | | 1.0% TN | | | 19-21 | (.04%) | | |
| | | 2.0 BS | Brij | | 22-24 | | | |
| | | 2.0 TX | | | 25-27 | | | |
| | | No detergent | | | 28-30 | | | |
| | (1.1X) | | | | 31-33 | 3.64 | | |
| | (5X) → dilute 1/2.5 = .04% | | | | 34-36 | 2 | | |
| | 2 x R26 | 0.1% + Enz | | | 37-39 | | | |
| | 2 x TFI | 0.1% | | | 40-42 | | | |
| | 2 x Vent buffer | | | | 43-45 | | | |
| | 5ul | | | | | | | |
| | dil = 0.04%/ul | | | | 46-50 | 2 | | |

Reassay on 10
this page 2-3-95
152 3-9-95
167 4-4-95
36, 10 5-26-95
52, 10 5-27

Tag 5ul
31-55WS
20ul P55,7
10' 74°C, 10ul P55,7
spot 40ul on 6 FC
dilution used for dil buffer of 9-20-94 (see new stocks)

Test Rxn mix
EKBT 15ul 1-31-95:
no dil 2 46ul
1/125 2
EP9407 2
1/125 2
mix P.120
made new mix with stock shown in red on P120 and repeated experiment on 2-3-95 - results on next page (P122)

incorporation!
label 5ul of #12 (5X) into 12ul Tag dil buffer P55,7

| | | | |
|---|-----------------|-----------------|--------------------------|
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 | Date
2-1-95
2-3-95 |
| | | Recorded by | |

To Page No.

122

Project No. _____

Book No. Av2

TITLE

unit/ptRelative
to Tray

Tray = 5

u/x N

From Pr ...

| | | | | | | | |
|------|----|----------|---|------|--------|--|---------------|
| 1E | 1 | 8410.00 | } | 8819 | .037 | | .03 |
| | 2 | 9136.00 | | | | | |
| | 3 | 8912.00 | | | | | |
| | 4 | 7465.00 | } | 7552 | .033 | | .03 |
| 2E | 5 | 8664.00 | | | | | |
| | 6 | 7728.00 | | | | | |
| | 7 | 7737.00 | } | 7580 | .032 | | .03 |
| 3E | 8 | 7235.00 | | | | | |
| | 9 | 7769.00 | | | | | |
| | 10 | 7579.00 | } | 6878 | .029 | ✓ | .02 |
| 4E | 11 | (3001)00 | | | | | |
| | 12 | 6178.00 | | | | | |
| | 13 | 7484.00 | } | 7812 | .033 | | .03 |
| 5E | 14 | 7833.00 | | | | | |
| | 15 | 8119.00 | | | | | |
| | 16 | 6228.00 | } | 6566 | .027 | ✓ | .02 |
| 6E | 17 | 6715.00 | | | | | |
| | 18 | 6755.00 | | | | | |
| | 19 | 8215.00 | } | 7824 | .033 | | .03 |
| 7E | 20 | 8743.00 | | | | | |
| | 21 | 6514.00 | | | | | |
| | 22 | 7996.00 | } | 8413 | .035 | | .03 |
| 8E | 23 | 8661.00 | | | | | |
| | 24 | 8581.00 | | | | | |
| | 25 | 7644.00 | } | 7533 | .031 | | .03 |
| 9E | 26 | 6981.00 | | | | | |
| | 27 | 7976.00 | | | | | |
| | 28 | 4900.00 | } | 4989 | .021 | } no detergent looks low. Can try
+ detergent in unit assay | .02 |
| 10E | 29 | 4647.00 | | | | | |
| | 30 | 5419.00 | | | | | |
| | 31 | 7509.00 | } | 7702 | .032 | | .03 |
| 11E | 32 | 6923.00 | | | | | |
| | 33 | 8674.00 | | | | | |
| | 34 | 8196.00 | } | 8075 | .034 | | .03 |
| 12E | 35 | 7970.00 | | | | | |
| | 36 | 8060.00 | | | | | |
| | 37 | 8015.00 | } | 7442 | .031 | | .03 |
| 3E | 38 | 7358.00 | | | | | |
| | 39 | 6954.00 | | | | | |
| | 40 | 8055.00 | } | 8479 | .035 | | .03 |
| 4E | 41 | 8359.00 | | | | | |
| | 42 | 9023.00 | | | | | |
| | 43 | 7844.00 | } | 7611 | .032 | | .03 |
| 5E | 44 | 7351.00 | | | | | |
| | 45 | 7638.00 | | | | | |
| | 46 | 9312.00 | } | 9580 | (0.04) | | .04 |
| | 47 | 9496.00 | | | | | |
| | 48 | 9290.00 | | | | | |
| | 49 | 9726.00 | } | | | | by Definitive |
| | 50 | 10073.00 | | | | | |
| | 51 | 58661.00 | | | | | |
| ativ | 52 | 60427.00 | } | | | | |
| | | | | | | | |

ave = 59544 \Rightarrow 1,478,600 cpm/50 \times 12 \times 2

37.2 cpm/pmol

To Page No

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Date

2-3-95

11/9/95

161

plates / 6.4, 8, 10.5, 20, 29 kb.

Page No. _____

Purpose: ~~to~~ make more cleaner plates of 6.4, 8, 10.5, 20, 29 kb.

plated quite a few for each at diff. conditions

these were grown under stringer conditions in the presence of Tel + Kan.

Read Amp plates, left at 37°, overnight.

6.4, 8.0 - colonies were small, especially 6.4

same each time whenever tried. - don't remember how

first looked ok. - well spread out bigger colonies. they were last time

streak them all at 4°.

picked a few single colonies from 6.4 & 8.0
grew them again for fresh minipreps. grown under
Kan + Tel conditions.

from 8.0 - did minipreps by alkaline lysis method, resuspended in
15 µl 95% x12 = 180 µl Total.

6.4 kb streak at 4°.

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11/11/95

Project No. _____
 Book No. _____

(see P 80)
 TITLE ^{32}P 23mer depatation reaction cond

126

From Page No. tube # 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

SX mixes # 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
 P. 125 4 μ l \rightarrow ✓ ✓ 4 4
 (0.15 pmol / μ l) \times 0.267 pmol primer \rightarrow ✓ - -
 (mixed as P75)
 Vent pol 2 μ l \rightarrow 2 2
 0.01 u/ml
 diluted in Vent storage
 w/ dilution buffer
 from NEB
 50% glycerol 2.0 μ l \rightarrow ✓ ✓ 2.8
 H₂O 17.4 \rightarrow 10.6 \rightarrow ✓ ✓ 4 1.2
 V_f = 20
 70°C 30'
 add 10 μ l cycle seq stop
 * ^{32}P 23mer 10 10 λ
 if 20%

(13 Rxns) cocktail
 (A) (B)
 ^{32}P 23 7.8 λ 7.8 λ
 H₂O 174.2 137.8
 50% glycerol 36.4
 V_f = 182
 182 0.01 unit Vent at 100,000 u/mg \approx
 add H₂O / tube # 1-10 # 11-20
 and 23 which gets 2 μ l of Vent dil buffer
 $\Rightarrow \approx 0.1$ pmol pol / 1 unit
 $= (0.002$ pmol pol total pol)
 $\frac{0.16$ pmol primer
 $\frac{0.002$ pmol pol
 $\frac{0.38$ pmol circles / 0.002 pmol pol = (192 circle / pol)
 (20% with a 23mer connected)

* ^{32}P 23-mer 19
 0.1 u Tris 0.1 μ l .3
 ^{32}P 23 (0.267 pmol primer / λ) P75 0.6 μ l 1.8 0.47
 193 m p 18 + 0.2 μ g / λ 2.5
 $= 0.084$ pmol / μ l 9.3 27.9 2.78 pmol circles
 19 μ l 30 50°C, 5' cool down

ig No. 1/5 but dil buff

Vent dil buff

50 mM Tris HEPH 7.4
1 mM DTT
0.10% NP40, Tween 20 each
50% glycerol
100 mM K-Cl

dilute 1/5
with 10 mM
Tris HEPH 7.5

1/5 Vent dil buff
CF

20 mM
0.2 mM
.02
100%
10 mM

Ran 80% gel with poor resolution,
run 16% PAGE plus new reactions on 2-13-95

16% PAGE see P 144, 1

24 25 26 27 28 29 30 31 32 33

| | | | | | | | | | | |
|--------------|-----------------------------|------|------|------|-----|-----|------|------|-----|-----|
| ant buffer | 2 | 2 | | | | | | | 2 | ✓ |
| 1, P125 | | | | | | | | | | |
| ant buffer | | | 4 | 4 | | | | | | ✓ |
| avg 190-83 | | | | | 4 | 4 | | | 4 | ✓ |
| 20 mM | | | | | 2 | 2 | | | 2 | ✓ |
| #10 P125 | | | | | | | 4 | 4 | | ✓ |
| Cheng mix | | | | | | | | | | ✓ |
| m (P126) | 0.6 | | | | | | | | | ✓ |
| 19 (P126) | 0.077 pmol circ / λ | | | | | | | | 5 | 5 |
| 0.01 μ A | 2 | 2 | | 2 | | 2 | | | | |
| 0.1 μ A | | 2 | | 2 | | 2 | | 2 | 2 | 2 |
| glycerol | | | | | 2.8 | | | | 2.8 | ✓ |
| | 15.4 | 15.4 | 13.4 | 13.4 | 8.6 | 8.6 | 13.4 | 13.4 | 11 | 4.2 |

✓ Cp 2 mM
✓ Cit has Mg, Okazaki
0.038 pmol circ total
my Cheng has on 1% glycerol at 1X need 7% more

$V_f = 20 \mu$

70°C, 12'

40% Acrylamide 200g
0.8% Bis 4g
H₂O

16% PAGE
start 1700 V at 1:45 pm
at 25 V, 15 mAmp

get ~ 7.4 cm/hr
need 3 hr

1-10 11-20 pri 21, 22 24-32 pri 10 empty
space space space big plate

went to 30 with constant set 2200-2250 V
8.7 cm/hr
To Page No. _____

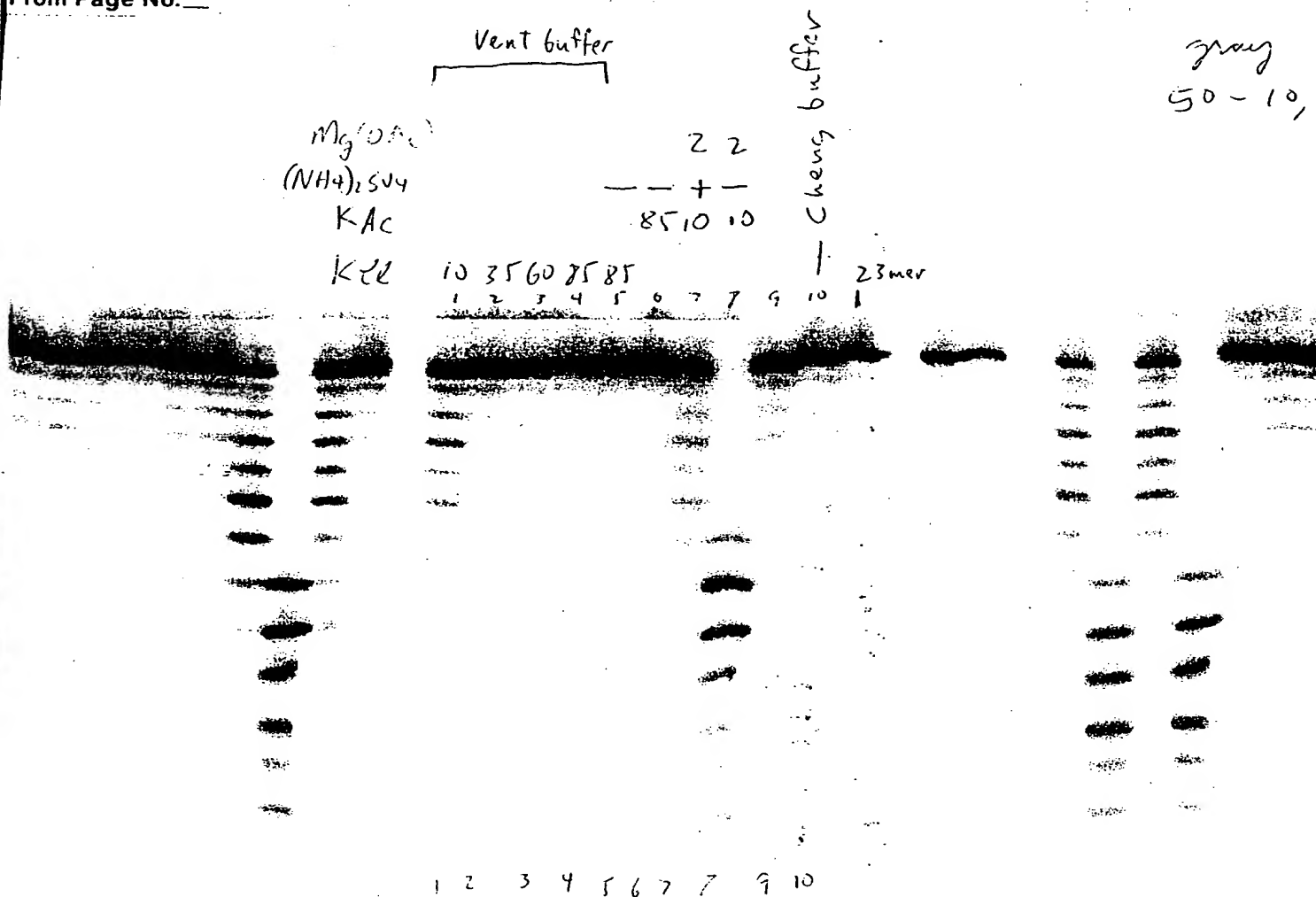
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Book No. _____

TITLE _____

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Results:

- #1, 10 KCl + $MgSO_4$ is same as KOAc, $MgOAc$ - get degradation if $K \leq 1$
- 1-5 increasing ionic strength eliminates degradation. #5 also 85 mM KAc same as KCl 85 mM
- 8 leave out $(NH_4)_2SO_4$ get best result degradation of all (don't have (-) $(NH_4)_2SO_4$ for 10 mM KCl and $MgSO_4$ only 85 mM this result also consistent with ionic strength effect
- 9 substitute tricine for Tris in Vent buffer has no effect
- 10 complete Cheng buffer - no degradation can be fully explained as due to 85 mM KAc - see # 4, 5 - 85 mM KCl or KAc no degradation in Vent buffer

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2/16/95

Record d by

2-13-95

- 02/14/95 - 06:45 pm

1.00x Counts

49.97



10000.00 D

Vent
 buffer
 NEB RL 5x G-TM RL
 .02 .2 .02 .2 .02 .2 .02 .2 .2 .2
 Cheng
 Vent (NEB) 23-mp/19
 Cheng G-TM 23-mp/19
 23 mer
 :Units
 Vent

23 mer is strongly
 protected when annealed.
 to m13 ssDNA

To Page No. _____

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suave a Polars

130 Tne vs Toy Project No. _____ Book No. _____ TITLE effect of Kcl on pol on M17 and primer degradation

From Page No. _____

| | (1) | (2) | (3) | (4) | (5) | (6) | |
|--|--------------|--------------|--------------|--------------|--------------|--------------|-----------------|
| Kcl mM | 50 | 75 | 100 | 50 | 75 | 100 | |
| 10 X Tag PCR buff | 8 μ l | 8 μ l | 8 μ l | 8 μ l | 8 μ l | 8 μ l | ✓ ✓ |
| Kcl 0.5M | 4 | 8 | | 4 | 8 | | ✓ ✓ |
| 32 P 23. mer (0.064 pmol $\frac{23. \text{mer}}{\lambda}$) | 8 μ l | 8 μ l | 8 μ l | 8 μ l | 8 μ l | 8 μ l | ✓ ✓ (0.064 pmol |
| 10 mM 4dNTPs | 10.6 μ l | 10.6 μ l | 10.6 μ l | 10.6 μ l | 10.6 μ l | 10.6 μ l | ✓ ✓ 200 |
| MgCl ₂ 50 mM | 2.4 μ l | 2.4 μ l | 2.4 μ l | 2.4 μ l | 2.4 μ l | 2.4 μ l | ✓ ✓ 1.5 |
| Tag 0.4 u/l | 2 | 2 | 2 | 2 | 2 | 2 | 0.8 |

Tne 0.8 u/l H₂O 58 54 50 58 54 50 ✓ ✓ 1.6 0.2 u

preheat tube to 70°C, start with 2 μ l pol VP = 10 μ l

remove 10 μ l at 1, 2, 5, 10 min to 5 μ l cycle seq stop

* rTag EKBT1 1-31-95 5 u/l } both diluted in Tag dil buffer
Tne 5 u/l A. Goldenum

32 P 23 mer same as P.75 (0.267 pmol 23 mer / λ)

32 P 23. mer

32 P_{primer} 0.267 pmol 23 mer / λ 15.8 μ l (4.2 pmol 23 mer tr

M13 mp19 0.2 μ g / λ 50 μ l (4.2 pmol circle
(0.084 pmol circle / λ)

1 mTas 7.5 0.6 66 μ l 0.064 pmol 23.1
use 1 μ l / 10 μ l re

To Page 1

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Deena aBslamp

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2-15-95

Primer degradation (see P80)

Project No. _____

Block No. _____

131

| ag N | 25-27 | 28-30 | 31-33 | 34-36 | 37-39 | 40 | |
|----------------|-------|-------|-------|-------|-------|------|--|
| vent | 8 | 8 | 8 | 8 | 8 | 8 | Does DMSO have any effect on contaminant? |
| Tag PCR buffer | | | | | | | |
| DMSO | | 1.6 | | | | 1.6 | C _f = 2.0% DMSO |
| 23 min | 1.91 | | | | | | ✓ (-0.64 pmol 23 min / 10 μl) (= 6.4 μM primer) |
| 1/2 5 min | | | 2.4 | | | | ✓ (note Vent buffer has 2 min Mg ²⁺) |
| 5 min | | | 4 | 8 | | | ✓ |
| 0.7 μl | 2 | | | | | | |
| 0 | 6.6 | 64.4 | 69 | 63.7 | 59.7 | 55.7 | 66.5 ✓ |

heat to 70°C, remove 10 μl at 2, 5, 15

15 min only, take 4 μl

pol/circles

0.1 unit Tag = 0.005 pmol (per 10 μl Rxn)

0.064 pmol 23 min / 10 μl (= 0.464 pmol at 10 μl)

pmol circles / pmol pol 0.012 3 ends / pol molecules

Expected units

0.1 u Tag gives 1 nmol at 30'

have 0.464 nmol at 10 μl reaction volume

so need ~14 min to replicate all DNA at least based on units - (but not sure M13 gives same units) 1 min would be ≤ 500 at extension at unit value rate

compared to PCR

Tag/time

1. This would be 0.5 units / 5 μl PCR

2. 6.4 μM primers (so 10 x less than 100 μM primers.)

T Page No. _____

Used & Understood by me,

Veronica Polansky

Date

2/16/95

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Recorded by

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2-15-95

132

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Book No. _____

TITLE _____

From Page No. _____

100 bp ladder cut 10072-015

10 μ l H₂O (vortex)

1 μ l 10 mCi/ml ³²P dCTP

15' 37°C \rightarrow 10 \times 0.2 m EDTA

get total $> 10^7$ cpm

load 0.2 μ l

(20 \times total) (20 bands)

10^7 cpm \rightarrow 5000 cpm/band

after 10 μ l EDTA

put 20 μ l
10 μ l
30 μ l

(Rxn + EDTA)
cycles seq stop

\geq 300,000 cpm/ μ l
10,000 cpm/band/ μ l

30 load 1 μ l

To Page N

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2-18-95

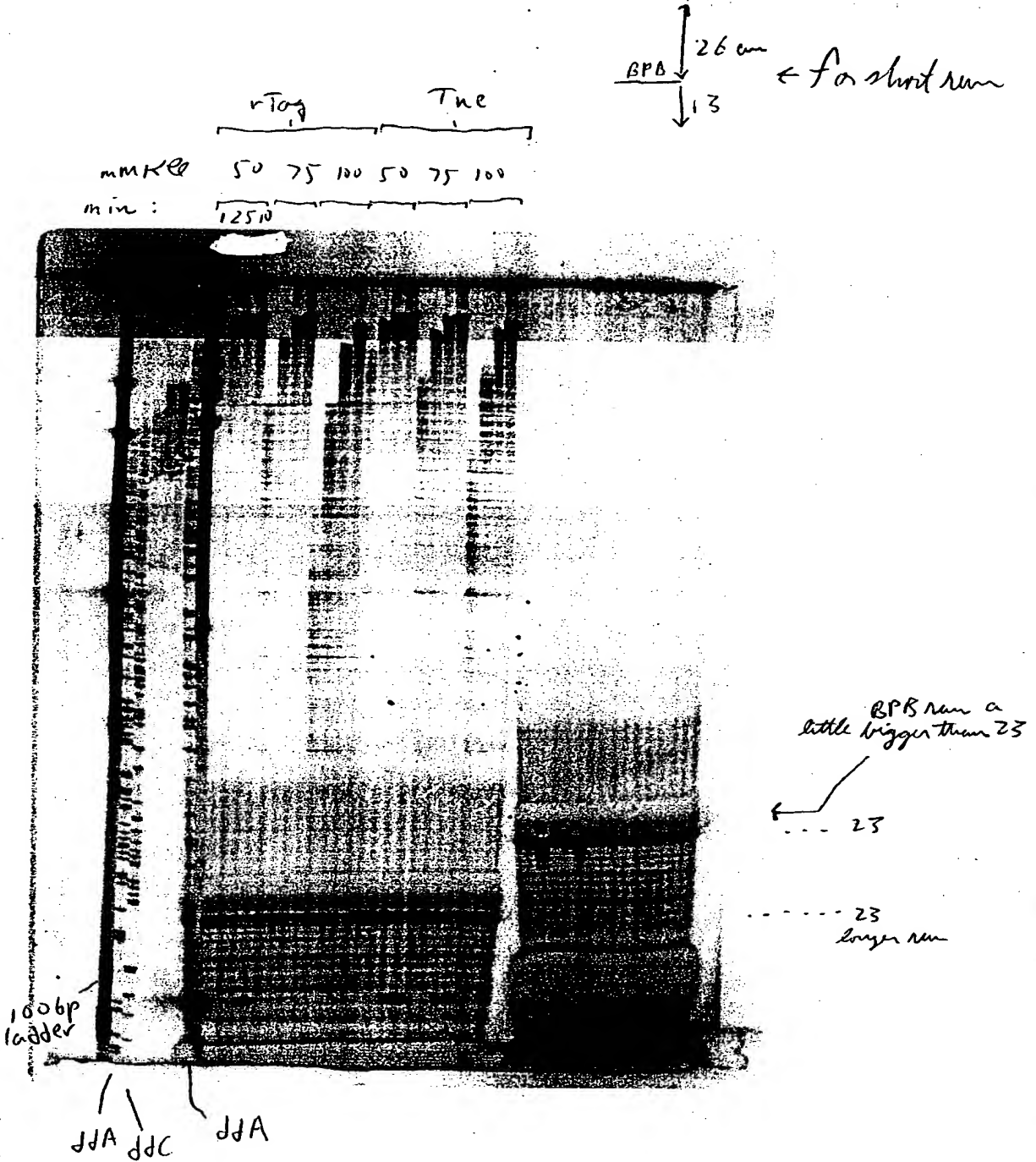
134

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Book No. _____

TITLE _____

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Project No. _____

Book No. _____ TITLE _____

136

From Page No. _____

primer 560826 of 8 called "AC" (23 mer with terminal A instead of G)

74.6 n mol total
746 x H₂O

Cp = 100 pmol primer / μ l (= 100 μ M prim)

Kinase

| | | | | | |
|---|-----------------------------|------------|---|---|--------------|
| 23 mer "AC" | (100 μ M primer) | 2 μ l | ✓ | ✓ | 200 p |
| 5x Kinase buffer | 100 pmol 23 mer / 1 | 8 | ✓ | ✓ | 23 ~ |
| ³² P γ ATP 10 mCi / μ l | (3.3 μ M ATP) | 20 | | ✓ | 66 p |
| PNK 1 u / μ l | | 2 | | | |
| H ₂ O | | 7 | ✓ | ✓ | |
| 30' | 37°C \Rightarrow 60°C, 5' | 40 μ l | | | Cp = 5 μ |

use 2 μ l / 50 μ l PCR
for 200 nM primer

note 1 unit T4 Kinase converts 1 n mol ATP / 30' at 37

To Page N

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3/16/95

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[Signature]

Date

2-20-95

| ge N | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | | |
|-----------------------|--------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|---|------------------------|
| XTag PCR buffer | 5 μ l | | | | | | | | | | | | ✓ | |
| "AC" P136 (5 μ l) | 2 μ l | | | | | | | | | | | | ✓ | (cf = 0.2 μ M 230) |
| 20 0.5 M | - | 1 | 2 | 3 | 4 | 5 | - | 1 | 2 | 3 | 4 | 5 | ✓ | |
| ne 5 μ l | | | | | | | | | | | | | | |
| dilute to 2 μ l | 2 μ l | | | | | | | | | | | | | (4 units total) |
| 7.5 to 20 μ l | | | | | | | | | | | | | | |
| water to 0.2 μ l | | | | | | | | | | | | | | (0.4 units) |
| Agell 2 50 mM | 10.5 μ l | | | | | | | | | | | | ✓ | (cf = 1.5 mM) |
| H ₂ O | | | | | | | | | | | | | ✓ | |
| | 39.538 | 39.538 | 39.538 | 39.538 | 39.538 | 39.538 | 39.538 | 39.538 | 39.538 | 39.538 | 39.538 | 39.538 | | |
| | 50 μ l | 50 μ l | 50 μ l | 50 μ l | 50 μ l | 50 μ l | 50 μ l | 50 μ l | 50 μ l | 50 μ l | 50 μ l | 50 μ l | | |

m M Kell cf = 50 60 70 70 90 100 50 60 70 70 90 100

70°C, remove 10 μ l to 5 μ l stop at 20, 60, 120 min

Results on P135

To Page No.

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Date

22/1/95

3/16/95

Recorded by

2-21-95

138

Project No. _____

Book No. _____

TITLE 33 mismatches and mismatched

From Page No. _____

11.64 nmol "33 correct"
(primer # 5381 DG1 (G01))

582 μ l H₂O

has correct G at 3' end at
of P1 site in MCS G-3'
GAATTC
20 μ M primer

13.42 nmol
33 mismatches

671 μ l H₂O

20 μ M primer

33 correct 1 μ M \times 5.3 \checkmark \checkmark (5.3 pmol total)
(1 pmol/1)

33 mismatches 1 μ M 5.3 \checkmark \checkmark
(1 pmol/1)

YADP (1.67 μ M ATP) \times 4 4 \checkmark \checkmark \checkmark (6.68 pmol)
5X Kinase buffer \times 4 4 \checkmark \checkmark \checkmark

PNK 1 μ l 1 1 \checkmark \checkmark \checkmark
H₂O \times 5.7 5.7 \checkmark \checkmark

20 μ l 20 μ l 37°C, 30' \Rightarrow 5'

1 pmol

2.108 pmol circle mp19 0.2 μ g/l \times 36.7 36.7 0.3 pmol primer

1M Tris pH 7.5 \times 0.6 0.6 \checkmark 0.6 pmol circles

20 μ l 66 μ l circle/1
5', 95°C cool slow = 2

use 2 \times / 20 μ l reaction

= 0.6 pmol primer in 20 μ l

To Page 1

Witnessed & Understood by me,

Demetri Polaris

Date

3/16/95

Invented by

[Signature]

Date

2-23-95

Recorded by

GEL

- 02/24/95 - 01:10 pm

0.66x Counts

0.11

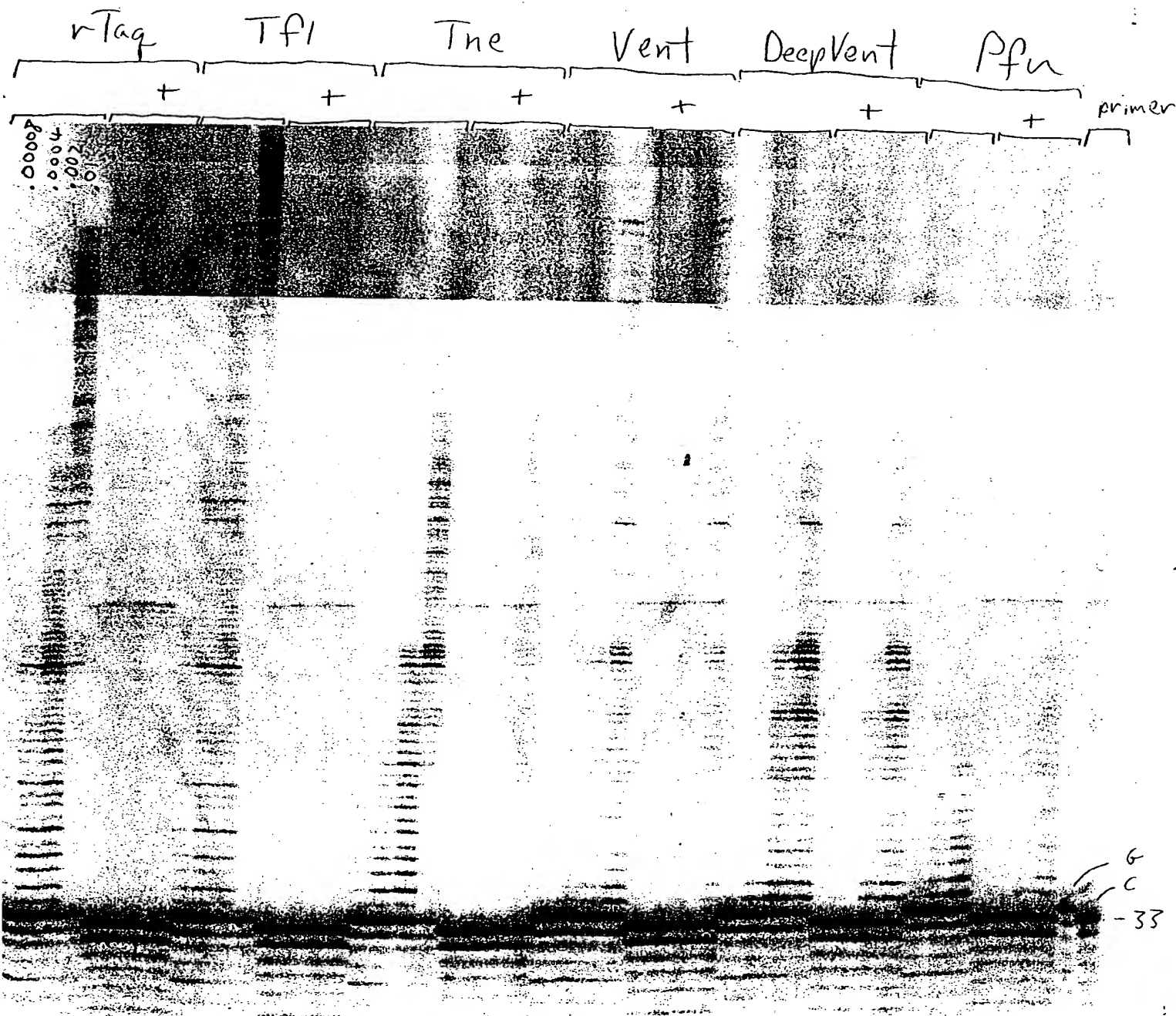


2000.25

D

Exhibit 47

Appl. No. 09/558,421



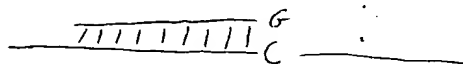
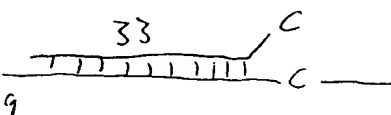
circles/pol molec

56

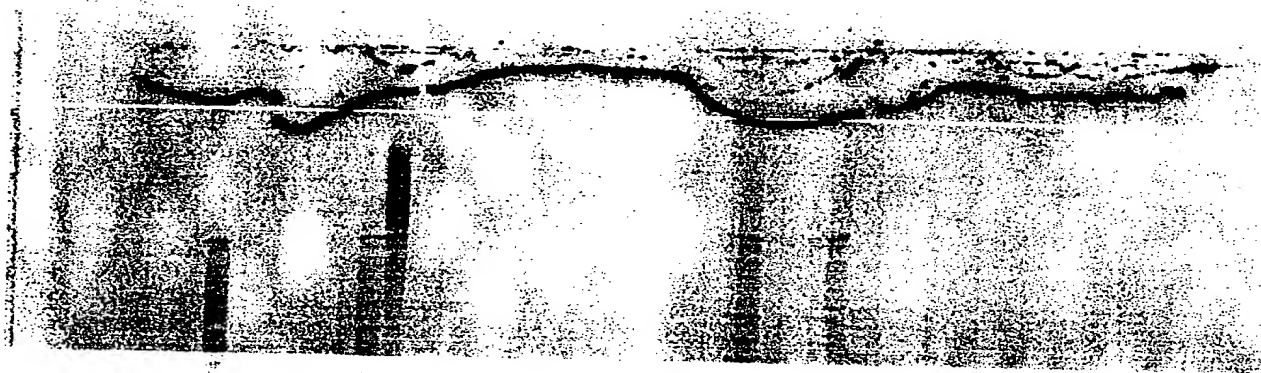
282

1410

7050



16% PAGE 33 watts, 4 1/2 hr
 XC went 21.7 cm ($R_f = \frac{21.7}{39} = 0.56$)
 33 mer migrated 25 cm
 XC runs as a 40 mer



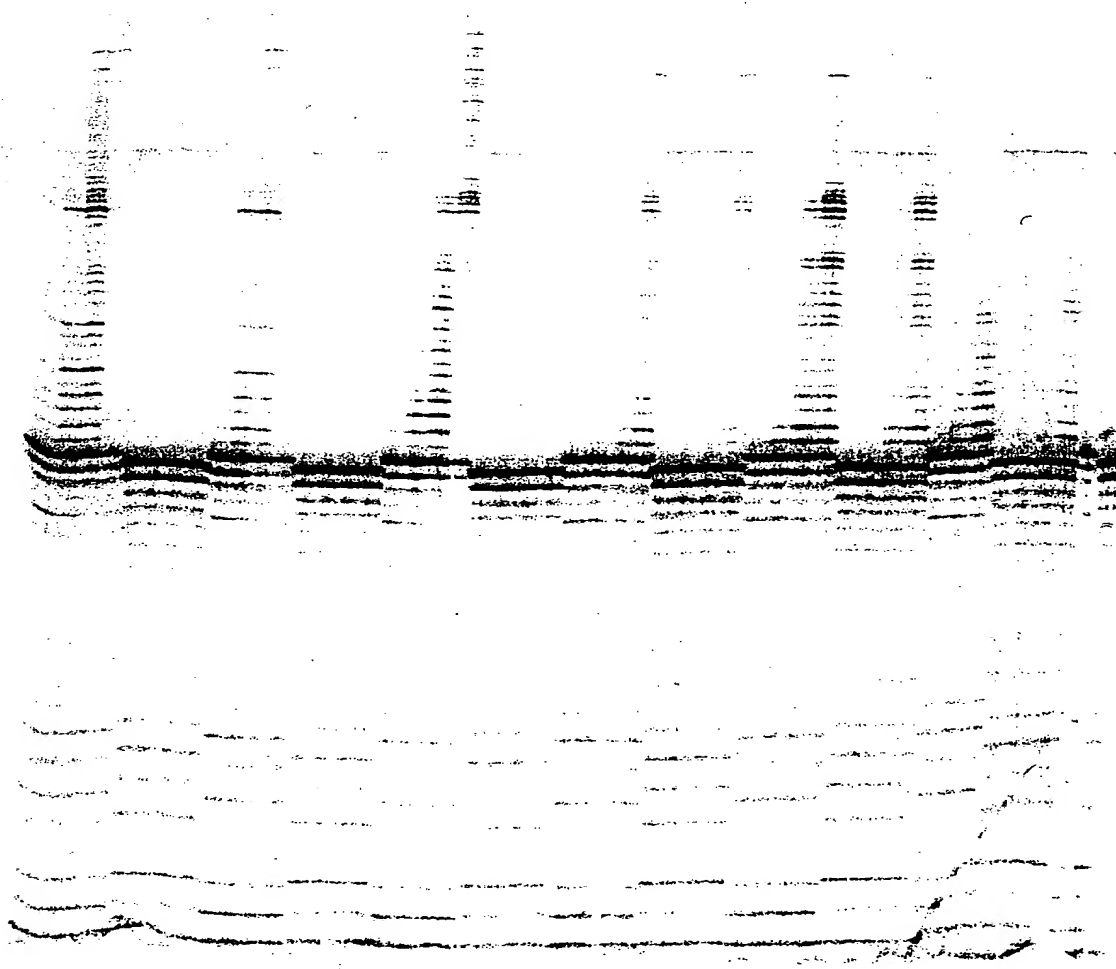
was
 in as 39 mer)
 XC →

3 →

XC →

33 mer →

16% →



could run XC to ~ 30 cm

ll. 33 mer went
 25 cm of 39 cm
 gel long the

2-7444

Project No. _____

Book No. _____

TITLE _____

140

From Page No. _____

 32 P correct. m.p. 19 (P. 138) 32 P mis. m.p. 19 (P. 138)

10 mM dNTPs each

50 mM MgCl₂

10x PCR buffer

10x Vent buffer

10x Pfu buffer
H₂O

rTag .00008 u/l

-31-83 .0004

.002

.01

Tfi .00008

Pfu .0004

130000A .002

.01

Tne .00008

.0004

.002

.01

Vent .00008

.0004

.002

.01

DeepVent .00008

.0004

.002

.01

Pfu .00008

.0004

.002

.01

To Page 1

Witnessed & Understood by me,

Deena Bobay

Date

3/16/95

Invented by

Recorded by

Date

2-24-95

| g | N | Vent | Deep Vent | | | | | | | | | | Pfu | | | | | 1st
vent | 2nd
vent | 3rd
vent | 4th
vent | | | |
|----|----|------|-----------|----|----|----|----|----|----|----|----|----|-----|----|----|----|----|-------------|-------------|-------------|-------------|-----|-----|----|
| 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | | | |
| → | | | | 2 | | → | | | | 2 | | → | | | | | | ✓ | | 28 | | 20 | 12 | |
| | | 2 | | → | | | | 2 | | → | | | | 2 | | → | | ✓ | | | 28 | | 20 | 12 |
| → | | | | | | | | | | | | | | | | | | ✓ | 5.6 | 5.64 | 4.24 | 2. | | |
| | | | | | | | | | | | | | | | | | | ✓ | 8.4 | 8.4 | - | - | | |
| | | | | | | | | | | | | | | | | | | ✓ | 28 | 28 | | | | |
| → | | | | | | | | | | | | | | | | | | ✓ | | | 20 | 20 | | |
| → | | | | | | | | | | | | | | | | | | ✓ | | | | | | |
| → | | | | | | | | | | | | | | | | | | ✓ | | | | | | |
| → | | | | | | | | | | | | | | | | | | ✓ | | | | | | |
| → | | | | | | | | | | | | | | | | | | ✓ | 196 | 196 | 146 | 146 | 146 | |
| → | | | | | | | | | | | | | | | | | | ✓ | 196 | 196 | 146 | 146 | 146 | |
| | | | | | | | | | | | | | | | | | | | 14 P.m. | 190 | 190 | 60 | 60 | |
| | | | | | | | | | | | | | | | | | | | use 190 | | | | | |
| | | | | | | | | | | | | | | | | | | | 20 P.m. | | | | | |

2 min 70°C
load with 10 µl after seq stop

note: 0.03 pmol 3' ends / Rxn
for 0.00008 units (lowest level):
 $(10 \times 10^9 \text{ nmol/min}) (0.00008 \text{ U}) = 0.03 \text{ pmol}$
30-min
micro
so expect only ~ 1 nt addition for
each primer in 1 min (based
on even distribution and process
of 1).

To Page No. _____

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Date

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Date

Carolina Polanco

3/16/93

Record d by

12-24 93

Project No. _____
Book No. _____

TITLE Repair of 3' mismatch
for TFI \pm Vent and rTog \pm DV, Pfu Tr

142

| From Page No. _____ | * 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|--|-----|---|---|---|---|---|---|---|---|----|----|----|----|----|----|-----|----|----|----|----|----|----|
| 32 P33 mis - m pl9
(P138 en. of 0.01 pm primer/1) | 2 | | | | | | | | | | | | | | | | | | | | | |
| 10 mM JNTPs | | | | | | | | | | | | | | | | | | | | | | |
| 5X Chemy complete (2/14/95) \checkmark 4 | | | | | | | | | | | | | | | | 0.4 | | | | | | |
| 5X elongase
H ₂ O | | | | | | | | | | | | | | | | 4 | | | | | | |
| TFI 0.1 μ l | | | | | | | | | | | | | | | | | | | | | | |
| 1 μ l | | | | | | | | | | | | | | | | | | | | | | |
| Vent 0.002 μ l | | | | | | | | | | | | | | | | | | | | | | |
| 0.01 μ l | | | | | | | | | | | | | | | | | | | | | | |
| 0.05 μ l | | | | | | | | | | | | | | | | | | | | | | |
| rTog 0.5 μ l | | | | | | | | | | | | | | | | | | | | | | |
| 5 μ l | | | | | | | | | | | | | | | | | | | | | | |
| Deep Vent 0.002 μ l | | | | | | | | | | | | | | | | | | | | | | |
| 0.005 | | | | | | | | | | | | | | | | | | | | | | |
| 0.05 | | | | | | | | | | | | | | | | | | | | | | |
| Pfu 0.002 0.005 | | | | | | | | | | | | | | | | | | | | | | |
| 0.005 | | | | | | | | | | | | | | | | | | | | | | |
| 0.05 | | | | | | | | | | | | | | | | | | | | | | |
| Tne 0.022 0.01 | | | | | | | | | | | | | | | | | | | | | | |
| 0.01 0.05 | | | | | | | | | | | | | | | | | | | | | | |
| 0.05 0.1 | | | | | | | | | | | | | | | | | | | | | | |

VF-202

Preheat all reaction to 70°C, start by addition of
3'P33 mis - m pl9, add 10 μ l cycles eq stop at 2 minutes

rTog, Tne TFI use Tog dil buffer
Pfu, Vent, Deep Vent use NEB Vent dil buffer

To Page N

Witnessed & Understood by me,

Deena Polanco

Date

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Inv nt d by

Rec rd d by

Date

2-27-95

15 Rxms

[illegible]

To Page No.

d & Understood by m ,

Date _____

Invented by**Date**

Rec rd d by

2-27-95

146

Project No. _____

Book No. _____

TITLE

 Δ KAc. Effect on pol and exo, The v

From Page No. _____

1 2 3 4 5 6 7 7 9 10 11 12 13 14 15 16 17 18 19 20

5x Mungy (no KAc
no DMSO
no Glycerol)
(at 5x = 100 mM Tricine pH 7.7,
5 mM Mg(OAc)₂)

✓ 4

KOAc 0.2 M

✓

0 1 2 3 4 5 6 7 8 9 10 0 1 2 3 4 5 6 7 8

*
0.33 correct. mPA (same as
p138 0.06 pmol circ./ λ)

✓ 2

*
32P 33 correct 5 μ M primer
(as was done for "AC" on 13.6)
10 mM 4 dNTPs
H₂O

✓ 0.4

✓ 11.6 10.4 9.6 8.8 7.4 6.6 5.6 4.6 3.8 2.6 1.6 11.6 10.4 9.6 8.8 7.6 6.6 5.6 4.6 3.8

vTag 0.001 μ / λ

2

Tne 0.004 μ / λ

2

Tne 2.5 μ / λ v_f = 20 λ

70°C, 5'

* 33 correct has
same 5' end as 23mer sequencing primer

To Page N

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Deborah W. Blazynski

Date

3/16/95

Invented by

Recorded by

Date

3-1-95

ag No. 27 28 29 30 31 32 33

→ ✓

2 3 4 5 6 7 8 9 10 ✓

→ ✓

12.2 11.2 10.2 9.2 8.2 7.2 6.2 5.2 4.2

JX Chung on P 79:

20 mM Tris HCl pH 8.7

1.2 mM MgOAc

80% glycerol

20% DMSO

plus K₂Cr₂O₇ which is varied

$C_p \approx 200 \text{ mM}$ from 0-100 mM in this experiment.

→

70°C, 60'

start 11.8

To Page No. _____

sed & Understood by me,

essence Polansky

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Dat

3-1-95

Project No. _____

10x PCR same as P140

Book No. _____

TITLE is Tne inhibitory at 7 units ?

148

From Page No. _____

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

10x Tqg PCR buffer ✓ 2

³²P 33mer. mp19 (P146) 2
(see P138)

50 mM MgCl₂ ✓ 0.6

10 mM JNTPA ✓ 0.4

H₂O ✓ 14

→ 13 → 14

r Tag:

0.25 u/l ✓ 1

0.5 u/l ✓ 1

1 ✓ 1

5 ✓ 4

5 ✓ .6

5 ✓ .8

5 ✓ 1

→ ✓

Tne

0.25 u/l - 1

0.5 1

1 1

5 4

5 6

5 8

5 1

- 1

1

1

4

6

8

1

Tqg storage buffer

✓ 0.6 .4 .2

✓ 0.6 .4 .2

1

✓ 0.6 .4

preheat to 70°C, add 2 ³²P 33mer. mp19 for 30 sec
kill with 10 μ l cyclo sig stop solution
with 10 mM extra EDTA → 0.0 Cf = 20 mM EDTA in stop

To Page No

Witnessed & Understood by me,
Diana Polarp

Date
3/16/95

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[Signature]

Dat
3-3-55

Project No. _____

Book No. _____

149

ig N _____

To Page No. _____

d & Understood by me,

Date

3/16/90

Invented by

Date

Record d by

see a Polamp

150

Project No. _____

Book No. _____

TITLE

AatII #1

³²P Kinase

From Page No. _____

see P136 for ↑ [primer]

10 μ M AatII #1
5x Kinase
3-²P ATP 10 mM
PNK 1 μ /1

5 ✓
4 ✓
1.0 ✓
1

(33 pmol ATP)

at 1x
5 mM MgCl₂ 55 mM
50 mM KCl

2 μ l

(\Rightarrow now with 2.5 μ M primer)

37°C, 30' \Rightarrow 80°C, 5'

↓

mix back into cold primer
at 5 cold to 1 hot primer

³²P AatII #1 2.5 μ M 13.3 20

(2.5 μ M)

cold AatII #1 16.6 25

(10 μ M)

30 45 μ l

(6.67 μ M)

(MgCl₂ = 2.2)

use 1.5 μ l / 50 μ l PCR for 200 nM
(adds 0.067 mM Mg₂ per PCR (f))

Aagob R. used in 14 PCR's

remove 10 μ l from each PCR to 5 μ l stop (up
and store at -20°C over weekend.

Result: Aagob R.
did PCR's with Inc

note smear (see E+Br stain
(P151 photo) is not hot.
2^o primer ("AatII #1") is not
needed for smear

To Page

Witness d & Understood by me,

Deborah Polak

Date

3/16/95

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Date

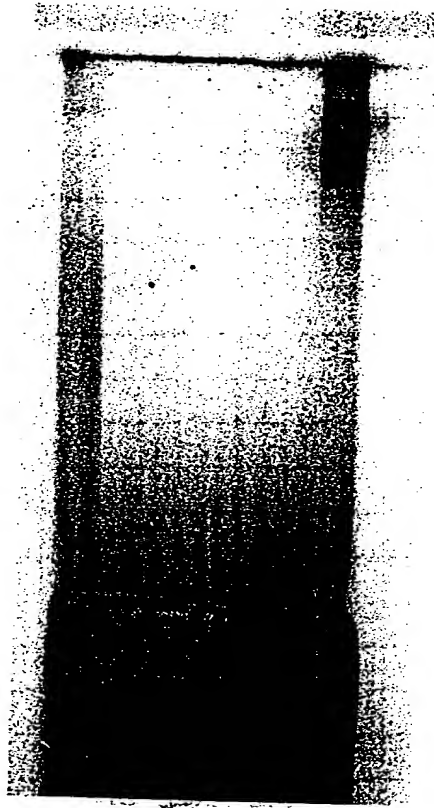
3-3-95

ig No. _____

100
1 2 3 4 5 6 7 8 9 10 11 12
1 1 2 3 3 4 4 5 6 6 7 7

0.5 x TBE

(lost 2nd, 5th)



To Page No. _____

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Date

3/16/95

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R cord d by

Date

2-7-95

Richard A. Pokany

Project No. _____

Book No. _____

TITLE _____

Unit assay for stability of vTag in
PCR mix. Repeat of assay on p 121

152

From Page No. _____

This assay is 33 days after the first assay of 2-3-95.

carry out all assays with exact same procedure
of P 120 - 122, same MgCl₂ TAPS, KCl mix of P12
same stock of 'activated' DNA, same 5' u/pl vTag stock

on P 121

(of 1-31-95)

(3' p d SP is a new stock of 10 mCi/ml on 3-10-95)

To Page N

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Deena Bolay

Date

3/16/95

Inv nted by

Record d by

Date

3-9-95
3-8-95

relatd to Tag \Rightarrow u
and $\frac{1}{1}$

Pr ject No. _____

B k No. _____

153

| activity | | | | | activity |
|----------|----|-----------|-------|-------|----------|
| idg #1 | 1 | 23165.00 | | | |
| | 2 | 26508.00 | 24896 | .031 | .037 |
| | 3 | 25014.00 | | | 84 |
| | 4 | 24738.00 | | | |
| 2 | 5 | 23608.00 | 24616 | .031 | .033 |
| | 6 | 25502.00 | | | 94 |
| | 7 | 23947.00 | | | |
| 3 | 8 | 24449.00 | 23577 | .030 | .032 |
| | 9 | 22336.00 | | | 94 |
| | 10 | 19450.00 | | | |
| 4 | 11 | 20001.00 | 19801 | .025 | .029 |
| | 12 | 19953.00 | | | 86 |
| | 13 | 21103.00 | | | |
| 5 | 14 | 20211.00 | 22158 | .028 | .033 |
| | 15 | 25159.00 | | | 85 |
| | 16 | 19309.00 | | | |
| 6 | 17 | 18318.00 | 18853 | .024 | .027 |
| | 18 | 18933.00 | | | 89 |
| | 19 | 22404.00 | | | |
| 7 | 20 | 25483.00 | 25532 | .029 | .033 |
| | 21 | 22108.00 | | | 87 |
| | 22 | 20542.00 | | | |
| 8 | 23 | 27602.00 | 25507 | .029 | .035 |
| | 24 | 21776.00 | | | 85 |
| | 25 | 22624.00 | | | |
| 9 | 26 | 23813.00 | 22051 | .028 | .031 |
| | 27 | 20017.00 | | | 90 |
| | 28 | 10829.00 | | | |
| 10 | 29 | 12483.00 | 11703 | .015 | .021 |
| | 30 | 11798.00 | | | (70) |
| Reaction | 31 | 23967.00 | | | |
| | 32 | 25056.00 | 24527 | .031 | .032 |
| | 33 | 24557.00 | | | 97 |
| | 34 | 26587.00 | | | |
| 12 | 35 | 23432.00 | 25000 | .032 | .034 |
| | 36 | 24980.00 | | | 93 |
| | 37 | 25401.00 | | | |
| 13 | 38 | 24104.00 | 24694 | .031 | .031 |
| | 39 | 24576.00 | | | 100 |
| | 40 | 25123.00 | | | |
| 14 | 41 | 25545.00 | 25962 | .033 | .035 |
| | 42 | 27217.00 | | | 94 |
| | 43 | 24143.00 | | | |
| 15 | 44 | 23491.00 | 25703 | .030 | .032 |
| | 45 | 23474.00 | | | 93 |
| | 46 | 30440.00 | | | |
| Tag | 47 | 31721.00 | 31731 | (.04) | |
| | 48 | 30572.00 | | | |
| | 49 | 32938.00 | | | |
| | 50 | 32985.00 | | | |
| 10 | 51 | 17357.00 | | | |
| det | 52 | 17994.00 | 17377 | .022 | |
| | 53 | 16781.00 | | | |
| 21 | 54 | 144943.00 | | | |
| 21 | 55 | 145358.00 | | | |

note #10 is not sufficient. so added det he
at .01 % from 20/10/4 each in Reaction mix

To Page No. _____

sed & Und rstood by me,

Date

Inv nted by

Date

R corded by

ance abolay

3/16/90

R

3-9-85

Test of rule to use 1/600 Tog dil
between 20-40 min after mixing

Pr j ct N _____
B ok N _____

Exhibit 53
Appl. No. 09/558,421

155

Standard Tog units array as per 120-120

10 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

e# 123 4 5 6 7 8 9
tube# 123 4 5 6

21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40

10 11 12 13
7 8 9 10 11 12 13

41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 70 2hr 3hr

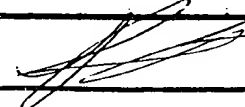
14 15 16 17 18
14 15 16 17 18

make a standard 1/600 dil^o

1797 μ l Tog dil buffer
3 μ l 5 u/l tog
Vortex 5"

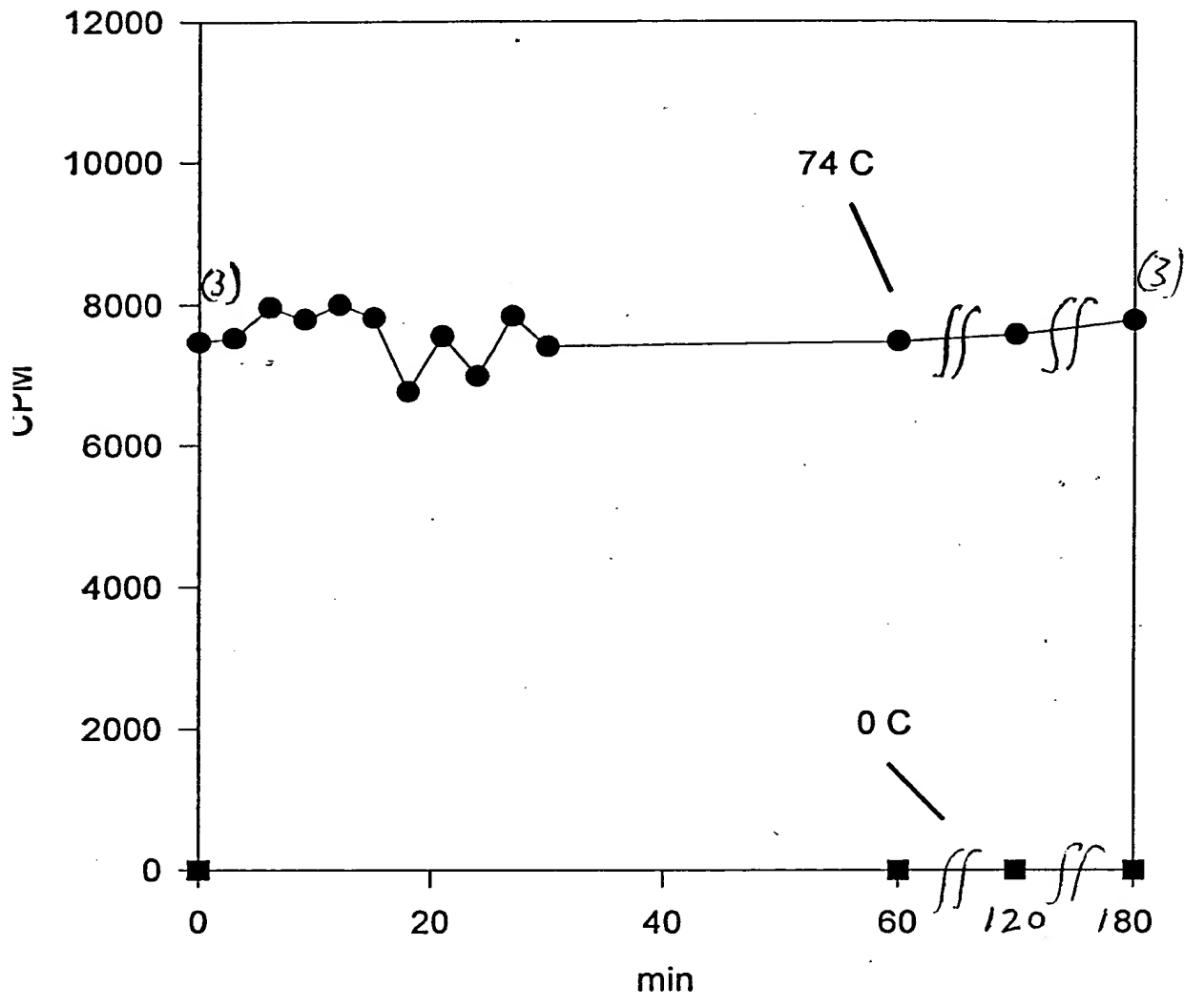
use immediately in triplicate for reactions 1, 2, 3 at
0, 20 sec and 60 sec
also # ~~1797~~ 20 sit on sec + 2 3 hr before 10)
EDTA (id no time at 74°C to see if any activity at 0°C.

To Page No. _____

| | | | |
|--|-----------------|---|-----------------|
| ed & Understood by me,
Masha Polansky | Date
3/16/95 | Invented by
 | Date
3-15-95 |
| | | Recorded by | |

Project No. _____
Book No. _____ TITLE _____

Time allowed before assay of Taq dilution



| SAM | CPM |
|-----|-----|
| 1 | 738 |
| 2 | 845 |
| 3 | 705 |
| 4 | 770 |
| 5 | 809 |
| 6 | 802 |
| 7 | 820 |
| 8 | 796 |
| 9 | 692 |
| 10 | 764 |
| 11 | 733 |
| 12 | 801 |
| 13 | 760 |
| 14 | 970 |
| 15 | 765 |
| 16 | 784 |
| 17 | 750 |
| 18 | 828 |
| 19 | 827 |
| 20 | 27 |
| 21 | 66 |
| 22 | 40 |
| 23 | 59 |

| | | | |
|---|----------------|-----------------------------------|-----------------|
| Invested & Understood by me,
<i>Deena Boland</i> | Date
4/4/95 | Invested by
<i>[Signature]</i> | Date
3-15-95 |
| | | Recorded by | |

Tet stock / streak T⁺ clones

Project N _____

Exhibit 54

Book N _____

Appl. No. 09/558,421

157

0.4g Tetracycline Sigma crystalline (not salt)
40 ml ETOH

Amp/Tet plates

have BBL Amp plates (100 µg/ml) Vol ~ 15 ml agar
to make 50 µg/ml Tet spread

15 µl 10 mg/ml Tet on each - let sink in > 30 min
25 µl 50 µg/ml Tet in 15 ml agar on plate

streak out cell (glycine) stocks of A.R.

sup 94-95 for stock names

grow at 30°C O/N

start 2 ml O/N from single colonies 3-21-95
of each in will grow, 100 µg/ml Amp, 50 µg/ml Tet

3-22-95

inoculate 0.4 ml of each O/N into 3.5 ml will grow
+ 100 µg/ml Amp, 50 µg/ml Tet

shake at 30°C starting at 8:30

To Page No. _____

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Date

Invent d by

Date

Lucia Polansky

4/4/95

Recorded by

3-20-95
3-21-95

Tet stock / streak T⁺ clones

Project No. _____

Block No. _____

Exhibit 55

Appl. No. 09/558,421

157

0.4g

Tetraogeline

Sigma crystalline

(not salt)

40 ml

ETOH

Amp / Tet plates

have BBL Amp plates (100 µg/ml) Vol. 15 ml agar

to make 50 µg/ml Tet spread

Typ. 10 mg/ml Tet on water - let sink in ≥ 30 min

25 50 µg/ml Tet in 15 ml agar on plate

streak out cell (glycerol) stocks of A.R.

sup 94-95 for stock names

grow at 30°C O/N

start 2 ml O/N from single colonies 3-21-95
of each in will grow, 100 µg/ml Amp, 50 µg/ml Tet

3-22-95

inoculate 0.4 ml of each O/N into 35 ml will grow
+ 100 µg/ml Amp, 50 µg/ml Tet

shake at 30°C starting at 8:30

To Page No. _____

Read & Understood by m ,

Date

Invented by

Date

Barbara Polansky

4/4/95

Recorded by

3-20-95

3-21-95

Tet stock / streak T+1 clones

Project N

Block No.

Exhibit 56

Appl. No. 09/558,421

157

Page N

0.4g

Tetracycline

Sigma crystalline

(not salt)

40 ml

ETOH

Amp/Tet plates

have BBL Amp plates (100 µg/ml) Vol ~ 15 ml agar
to make 50 µg/ml Tet spread

15 µl 10 mg/ml Tet on each - let sink in ≥ 30 min

20
50 µg/ml Tet in 45 ml agar on plate

streak out cell (glycerol) stocks of A.R.

SUP 94-95 for stock names

grow at 30°C O/N

start 2 ml O/N from single colonies 3-21-95
of each in will grow, 100 µg/ml Amp, 50 µg/ml Tet

3-22-95

inoculate 0.4 ml of each O/N into 35 ml will grow
+ 100 µg/ml Amp, 50 µg/ml Tet

shake at 30°C starting at 8:30

Page N

Read & Understood by m

Date

Invent d by

Date

Ernest Polansky

4/4/95

Record d by

3-20-95

3-21-95

From Page No. _____

3-22

30°C
Start 8:30
12:30 .274
2:00 .770

ASSD

↓ 42°C, 15 min
↓ 1 hr 37°C

3-23

extract w/ 55°C heat for FrI is
same as p 95 and p 115, 6

pol assay is same as PST except add just
2 µl FrI' / 97 µl Rxn cocktail
and remove three points
array 1 2 5 µl of ~~Rxn~~ FrI' in 50 µl
Taq unit array (using TFI buffer system)
for 5 min at 74°C

| | <u>3-23-95</u> | <u>12-15-94</u> |
|-------|----------------|-----------------|
| | % | |
| 106 | (100) | 64 |
| 107 H | 87 | 92 |
| 108 H | 86 | (100) |
| 152 | 83 | 59 |
| 151 | 56 | 95 |
| 202 | 20 | 26 |
| 109 | 2 | 11 |

To Page No. _____

Witnessed & Understood by me,

Deena a Polkamp

Date

4/4/95

Invented by

Rec rd d by

Date

3-23-95

Project No. _____

Book No. _____

TITLE _____

158

From Page No. _____

3-22.

30°C
Start 8:30
12:30 .274
2:00 .770

ASD
↓ 42°C, 15 min

↓ 1 hr 37°C

extract and 55°C heat for FrI is
same as p 95 and P115, 6

3-23

pol assay is same as PST except add just
2 µl FrI' / 97 µl Rxn cocktail
and remove time points
array 1 2 5 µl of FrI' in 50 µl
Toy unit assay (using TFI buffer system)
for 5 min at 74°C

| | 3-23-95 | 12-15-94 |
|------|---------|----------|
| 106 | (100) | 64 |
| 107H | 87 | 92 |
| 108H | 86 | (100) |
| 152 | 83 | 59 |
| 151 | 56 | 95 |
| 202 | 20 | 26 |
| 109 | 2 | 11 |

To Page No

Witnessed & Understood by me,

Deena a Pokany

Date



4/4/95

Invented by

Record d by

Dat

3-23-95

| | | | |
|---|---------------|---|-----------------|
| d & Und rst od by m ,
 | Dat
4/4/95 | Invented by
 | Date
3-23-95 |
| | | Recorded by | |

160

Project No. _____

Book No. _____

TITLE

5% PEI stock

From Page No. _____

Same as P155, 6 except ~~use~~ instead
of using complete Tag ext buffer (P167, 3)
just use 50 mM Tris HCl pH 7.5, 1 mM EDTA

A = 50 mM Tris pH 7.5 275 ml
1 mM EDTA

(55264A BRG) PEI 50% 50 ml

stir $\geq 30'$

adjust pH to 7.4 with HCl
add A to $V_f = 500$ ml

To Page 1

Witnessed & Understood by me,

Diana Bolamp

Date

4/4/95

Invented by

Recorded by

Dat

3-24-95

grow 2L TF1-106

Exhibit 59
Project No. _____
Book No. _____
Appl. No. 09/558,421

161

19 N

make 2x LB (il 40 g/L of LB broth base
eg as per P 119, b for D. + E

make 20 ml O/N of TF1-106
in LB + 100 µg/ml Amp, 30 µg/ml Tet
(Morgan uses 15-20 µg/ml Tet)

10 mg/ml

Ampicillin (Sigma A-9518)

2 g

H₂O

200 ml

filter sterilize

inoculate 10 ml O/N / 1L LB

start shaking at 30°C at 7:20 AM
start 7:20 AM
12:30 PM 0.567

induce each at 42°C, 15' - by rapidly
bringing up to 42°C in hot tap water bath
and then 42°C in water shaker 15'

37°C 1 hr in air shaker
cool in ice water bath

and 1 hr 31°C at 2:05 and 2:35 respectively

OD₅₅₀ final = 0.812 ⇒ recovered 5.64g cells
Spin 51L GSS 45 min

To Page No. _____

Read & Understood by me,

Date

Invented by

Date

Researcher's Name

4/4/95

Recorded by

3-26-95
3-27-95

Project No. _____

Book No. _____

TITLE _____

5 buffers for 50g TFI prep

162

Form Page No. _____

follow v Tag PRP 91342. PRP * except for a 2m KCl in buffer B.

2L
buffer B

1L
buffer C

1M Kphos monobasic ✓ x 34.2

17.1 ml

1M Kphos dibasic ✓ x 15.7

7.9 ml

glycerol ✓ x 160

80

KCl ✓ x 7.46g
(50mm)

149.12
(2m)

EDTA 0.5M x x 0.4ml

0.2 ml

BME 14.5M ✓ x 700 µl

350 µl

H₂O 2L

1L

buffer C is 2mK
here in order to do
elution point - m
in Tag PRP C.
700 mM KCl

To Page N

Witnessed & Understood by me,

Deanna Polanco

Date

4/4/95

Inv. nted by

Rec rd d by

Date

3-27-95

AmSO₄ optimization for TFI
(can see P 22, 7 for Tag)

Project No. _____

Exhibit 61

Appl. No. 09/558,421

B ok No. _____

163

ag N -

3.64 g

TFI cells (P161)

18 ml

Tag ext buffer (P167,3)

sonicate

heat treat 75°C, 30 min

PEI

adjust to 200 mM NaCl

Vol = 20 ml so add 1.33 ml NaCl 3M

add 5% PEI (P160) to C_f = 0.4%
stir 15 min (1.7 ml 5% PEI)

Centrifuge SS 34 15' 15 K

residual 17 ml

supr

= Fr I' / PEI

start 11:30 AM

stir AmSO₄ in 15', spin
SS 34 15 K, 15 min

2 AmSO₄

of
salt

| i | Fr I' / PEI | 2.45 g | 25 |
|----|-------------|--------|----|
| 2 | | .493 | 30 |
| 3 | | .51 | 35 |
| 4 | | .51 | 40 |
| 5 | | .527 | 45 |
| 6 | | .527 | 50 |
| 7 | | .544 | 55 |
| 8 | | .561 | 60 |
| 9 | | .561 | 65 |
| 10 | | .578 | 70 |

To Page No. _____

Used & Understood by me,

Date

Inv nted by

Dat

rebecca Polansky

4/4/95

Recorded by

3-29-95

Project No. _____

Book No. _____

TITLE

Pol assay of AmSO₄ supe

64

from Page No. _____

assay 2 μ l of $1/100$ dil of each supe in 48 μ l R_{xn} mix (P-120) for 15 min at 74°
kill with 10 μ l EDTA
spot 40 λ

| Bradford | | Ass | mg/ml | To Protein remaining |
|-----------------------|-------|------|--------|----------------------|
| I / PEI | 20 | | | |
| AmSO ₄ 25% | | .412 | (0.39) | 100 |
| | | .450 | 0.40 | 102 |
| 30 | | .430 | .37 | 97 |
| 35 | | .449 | .40 | 102 |
| 40 | | .425 | .37 | 97 |
| 45 | | .405 | .36 | 92 |
| 50 | | .370 | .33 | 85 |
| 55 | | .347 | .31 | 79 |
| 60 | | .340 | .30 | 77 |
| 65 | | .278 | .25 | 64 |
| 70 | | .242 | .21 | 54 |
| I-PEI/70% | 20/20 | .604 | | |
| BSA 1mg/ml | 1 | .105 | | |
| | 2 | .176 | | |
| | 4 | .263 | | |
| | 6 | .382 | | |
| | 8 | .474 | | |
| | 10 | .546 | | |

| AmSO ₄ % | CPM1 | μ /ml = 0.64 | |
|---------------------|-----------|------------------|---|
| 0 | 11483.00 | (100)% | \Rightarrow 10,980 μ /17ml FRI \Rightarrow 3017 $\frac{\mu}{g}$ cells |
| 25 | 10706.00 | 93 | (3.64% note used) |
| 30 | 11635.00 | 100 | |
| 35 | 10329.00 | 90 | |
| 40 | 7609.00 | 66 | |
| 45 | 803.00 | 7 | |
| 50 | 465.00 | 4 | |
| 55 | 514.00 | 4 | |
| 60 | 258.00 | 2 | |
| 65 | 313.00 | 3 | |
| 70 | 230.00 | 2 | |
| Blank | 126.00 | | |
| 21 | 106668.00 | | |

\Rightarrow 66.7 cpm

conclude 45% AmSO₄ brings down > 90% in

BSA

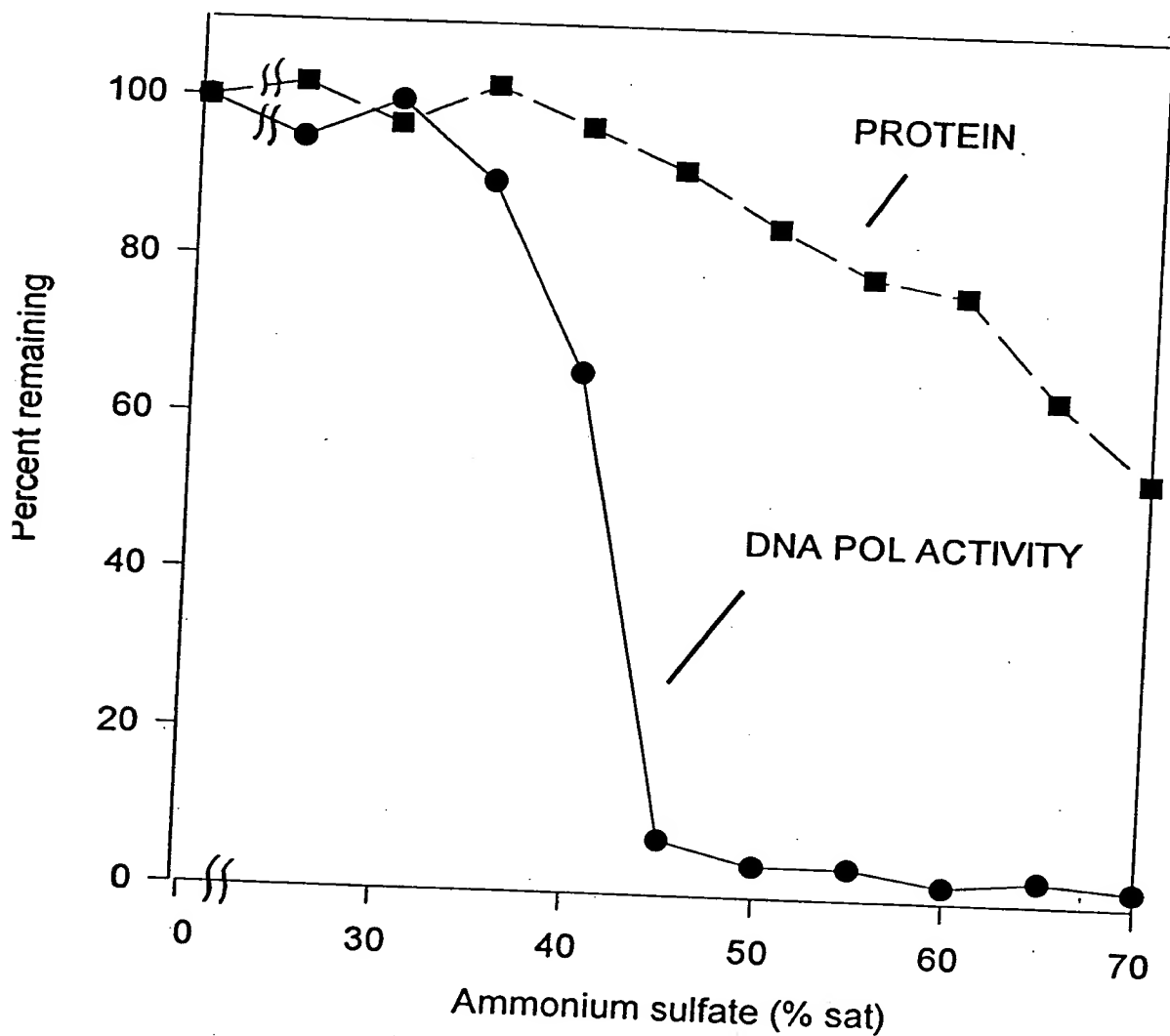
Project N

Bo k N

165

ig No

Precipitation of Tfl DNA polymerase



To Page No. _____

sed & Und rstood by me,

Dat

Inv nt d by

Date

Sue A. Borcup

4/4/95

Recorded by

166

Project No. _____

Book No. _____

TITLE prep a 180 ml sepharose 200

From Page No. _____ in a Mannin 2.6 XK

1. make slurry of cold sepharose 200 - want a
1.5 x vol of pack vol

3. 1.5 x 200 ml slurry = 300 ml

2. add 100 ml col buffer (buffer B p 162)
so vol now = 2 x pack vol

use reservoir and gravity flow (got 50 ml/min with effluent tube 2
below bottom of column and 100.
in reservoir) - ~ 1/4 vol vol/hr

bed volume ended up ~ 185 ml (2.6 cm x 35 cm)

5 well 25 ml (bed vol) of Blue sepharose (Ph
L65, in buffer B (p 162))

since dry swells 4 x 6.25 g

To Page No. _____

Witnessed & Understood by me,
Deborah Polansky

Date
4/4/95

Invented by

Record d by

Date

4/31/95

Stability unit assay for Tag
series as p 121 and 152

Project No. _____
Book No. _____

Exhibit 64
Appl. No. 09/558,421

167

No. _____

note stability study tube # 10 (unit assay # 27-30)
get 0.01% Taren 20/NP40 each added to reaction
by adding 0.5 ml of 1% stock

tube 51-56 =

Dextran

1.25 mg/ml

1 ml

Cf

0.025

2.5

1

0.05

5

1

0.1

10

1

0.2 mg/ml

10

2

0.4

10

3

0.8

51 19252.00
52 18303.00
53 18777.00
54 18582.00
55 17015.00
56 17487.00
57 267.00
58 104554.00

⇒ 65.3 cpm/pmol

To Page No. _____

ed & Understood by me,

reera Pokay

Date

4/13/95

Invented by

Recorded by

Date

4-4-95

Project No. _____

Book No. _____

TITLE _____

u/λ on P122

From Page No. _____

SAM

CPM1

u/λ assuming
rTag in .044/λ in 1/12581

oto

o/u/λ P122

| | | | | | |
|------|----|----------|-------------------------------------|-----------------|------|
| 1 | 1 | 13329.00 | .032 | .037 | 8604 |
| | 2 | 14243.00 | | | |
| | 3 | 14542.00 | | | |
| | 4 | 14132.00 | | | |
| 2 | 5 | 13839.00 | .032 | .033 | 97 |
| | 6 | 13367.00 | | | |
| | 7 | 14361.00 | | | |
| 3 | 8 | 14576.00 | .033 | .032 | 97 |
| | 9 | 14684.00 | | | |
| 4 | 10 | 11765.00 | | | |
| | 11 | 12054.00 | .027 | .029 | 93 |
| | 12 | 11446.00 | | | |
| 5 | 13 | 13666.00 | | | |
| | 14 | 13091.00 | .030 | .033 | 91 |
| | 15 | 12913.00 | | | |
| 6 | 16 | 10381.00 | | | |
| | 17 | 10049.00 | .024 | .027 | 89 |
| | 18 | 10787.00 | | | |
| 7 | 19 | 16428.00 | | | |
| | 20 | 14956.00 | .034 | .033 | 103 |
| | 21 | 15556.00 | | | |
| | 22 | 15357.00 | | | |
| 7 | 23 | 14468.00 | .033 | .035 | 94 |
| | 24 | 13489.00 | | | |
| 9 | 25 | 14348.00 | | | |
| | 26 | 12027.00 | .030 | .031 | 97 |
| | 27 | 13354.00 | | | |
| * 10 | 28 | 9416.00 | | .022 | |
| | 29 | 8913.00 | .021 | .021 | 100 |
| | 30 | 9177.00 | | | |
| | 31 | 13920.00 | .032 | .032 | 100 |
| 11 | 32 | 13672.00 | | | |
| | 33 | 13373.00 | | | |
| | 34 | 14628.00 | | | |
| 12 | 35 | 13728.00 | .033 | .034 | 97 |
| | 36 | 15178.00 | | | |
| | 37 | 14616.00 | | | |
| 13 | 38 | 14209.00 | .034 | .031 | 109 |
| | 39 | 15366.00 | | | |
| 14 | 40 | 14402.00 | | | |
| | 41 | 14584.00 | .034 | .035 | 97 |
| 15 | 42 | 15003.00 | | | |
| | 43 | 12819.00 | | | |
| | 44 | 13391.00 | .030 | .032 | 94 |
| | 45 | 13180.00 | | | |
| | 46 | 16169.00 | | | |
| Tag | 47 | 18733.00 | 17463 ave (.044/λ)
by definition | | |
| | 48 | 18552.00 | | | |
| | 49 | 16396.00 | | | |
| | 50 | 12907.00 | | | |

* for #10, use .022 u/λ on P153 for
data added as 0.022 u/λ point

To Page No

Witnessed & Understood by me,

Date

Invented by

Date

Deanna Polansky

4/13/95

Rec rd d by

4-4-95

| je No. _____ | P12 in
other point | 1 month | 2 months | 4 months |
|--------------|-----------------------|---------|----------|----------|
| .1% TN | | 84 | 86% | 87 |
| .2% BJ | | 94 | 97 | 98 |
| .2% TX | | 94 | 97 | 106 |
| .01% TN | | 86 | 93 | 93 |
| .02% BJ | | 85 | 91 | 105 |
| .02% TX | | 89 | 89 | 88 |
| 1% TN | | 88 | 103 | 104 |
| 2% BJ | | 83 | 94 | 91 |
| 2% TX | | 90 | 97 | 99 |
| No detergent | | 95 | 95 | 91 |
| 1.1x | | 97 | 100 | 94 |
| 5x | | 93 | 97 | 100 |
| 2x R2GE 0.1% | | 100 | 109 | (35) |
| 2x Tf 1.01% | | 94 | 97 | 89 |
| 2x Vent | | 93 | 94 | 97 |

To Page No. _____

I & Understood by m ,

aaa Polay

Date

4/13/95

Invented by

Recorded by

Dat

4-4-95

Project No. _____

Book No. _____

TITLE _____

Tf1 growth of 4-4-95

70

from Pag No. _____

got ~ 0.6-0.8 g cells from 50ml samples taken
at 0 1 2 3 4 hr post induction for 10L pen
of minimal media (001R) and the same for
buffered rich (002R)
plus 50ml at end (~4h post induction) for
(002R) plus 114g bulk
[chipped off 0.55g of bulk for 4hr 002R sample]

Resuspend cells in Taz ext buffer (P167, 3)
add 25 ml ext buffer \Rightarrow 0.9 g cells/ml

sonicate 3 x 10 sec max setting microtip

microport 15 min, supel = Fr I

90°C 5 min

microport 15 min - supel = Fr I'

22.2 cpm
pmol
P167

pol assay is 2 μ l of 1/100 and 1/500 dil
of Fr I'

| hr | cpm | pmol | μ /ml | mg/ml | mg |
|-----------------|-----|---------|-----------|-------|-------|
| after induction | | | | | |
| 0.50 | 1 | 874.00 | -21 | 0.31 | 0.548 |
| | 2 | 440.00 | | | 1.03 |
| 1 | 3 | 6172.00 | | | 306 |
| | 4 | 1538.00 | 2.6 | .677 | 1.28 |
| | 5 | 5174.00 | | | 2029 |
| 2 | 6 | 1058.00 | 1.8 | .648 | 1.23 |
| | 7 | 6330.00 | | | 1467 |
| 3 | 8 | 1537.00 | 2.6 | .670 | 1.27 |
| | 9 | 5734.00 | | | 2050 |
| 4 | 10 | 1206.00 | 2.1 | .662 | 1.25 |
| | 11 | 1058.00 | | | 1675 |
| C | 12 | 324.00 | 0.58 | .639 | 1.21 |
| | 13 | 3961.00 | | | 314 |
| 1 | 14 | 1227.00 | 2.1 | .672 | 1.29 |
| | 15 | 4250.00 | | | 1626 |
| 2 | 16 | 1009.00 | 1.7 | .700 | 1.33 |
| | 17 | 4730.00 | | | 1282 |
| 3 | 18 | 1046.00 | 1.8 | .641 | 1.21 |
| | 19 | 3435.00 | | | 1483 |
| 4 | 20 | 763.00 | 1.3 | .734 | 1.27 |
| | | | | | 1018 |

To Page No. _____

Witnessed & Understood by m ,

Deena Polay

Date

4/13/95

Inv nted by

Recorded by

Date

4-15-95

114 g *Thomomys f. l.*
mini g *gambelii*

Pr j ct No. _____ Exhibit 66
Book N . _____ Appl. No. 09/558,421

171

cells 9504-02-767-03-002R
(4 hr after induction)

Follow rTag PRP Document # 91342. PRP

114 gram cells

450 ml Tag extract buffer (buffer A)
with fresh ^{5 mM} BME + 50 µg/ml PMSF

~ 564 ml (30 ~ 0.2 g cells/ml)

one pass *Thomomys gambelii* 10,000 PSI

heat to 75°C ~~50~~ (~15')

in 90°C water bath

15 min more at 75°C → cool in ice slurry

Adjust NaCl to 200 mM

have 550 ml Fr I' (ie after heating)

add 6.43 g NaCl

PEI adjusted to 0.4% by adding

47.8 ml 5% PEI pH 7.4 slowly, then
stir 15 min more

To Page No. _____

ed & Understood by m ,

Date

Invented by

Date

seen a Polars

4/13/95

Recorded by

4-7-95

Spin 30 min in GSA 13,000 RPM

5 Ammonium sulfate

Recovered 506.6 ml of Fr I' / PEI
want 4.75% $\text{Am}(\text{SO}_4)_3$ saturation

$$= 295.5 \text{ g} / \text{L}$$

so add 149.7 g to 506.6 ml Fr I' / P

add slowly, stir 30 min more

centrifuge GS-3, 2500 rpm, 60 min

— $\text{Am}(\text{SO}_4)_3$ pellet was coming off side of bottle
after 60 min spin
looks like ^{density} pellet & ^{density} solution

will try 2 hr at 13000 RPM in GSA

27000 g compared to ~12000 for GS-3

and smaller bottles (~138 ml / bottle in 4 bottle
— result: pellets still floating

— collected ppts in filter and mixed into
32 ml of clear filtrate

— spin 30 min in SS-34 1PK

and spin 1 ml of 32 ml total in microfuge for
unit assay.

3 N —
Try diluting 1:1 the suspended AmSO_4 ppt
+ Try ext buffer lacking glycerol (ie 50 mM
Tris HCl pH 7.5, 10 mM KCl) plus 47.5%
saturated AmSO_4 . ie, the only effect is
to reduce Cf of glycerol from 8% to 4%
to see if ppt will pellet better

Result: ppt floats in 4% and also w/o glycerol!
it does ~~not~~ sink in H₂O

Result:
see P 176 — cells induced only 1 hr don't
have problem of AmSO_4 pellets not sinking
must be too many lipids in cells used here
from 4 hr fermentation time point!

To Page No. _____

d & Understood by m ,

Date

Invented by

Date

sue a. P. Camp

4/13/95

Recorded by

7-8-95

Project No. _____

Book No. _____

TITLE _____

Stability of Toy at room Temp

74

from Page No. — see P154, 3-15-95. Samples have been at room Temp 24
assay same as P121, 152.

0.5 M
T cps 200 ml pH 9.3 (at room Temp)
(243.3 mW) (Suzuna T^{cut#}-5130)

24.33g + ~140ml H₂O
2m KOH to pH 9.5

H₂O to 200 ml

tube # 1-30 is stability study 1E-15E in duplicate
note tubes 19, 20 (no detergent) gets 0.5ul of 1% Tween 20/R
24, 24 in the reactions.
(ie sample is stability study)

Witnessed & Understood by me,
Dorinda A. Polay

Date
4/13/95

Invented by
Recorded by

[Signature]

Date
4-11-95

To Page No

Results of P114
Crack TFI same as P

Project N _____

Book No. _____

175

| SAM | CPM1 | ave | $\frac{a}{1}$ | from TIME | $\frac{a}{b}$ |
|---------|-----------|-------|---------------|-----------|----------------|
| 1 | 26896.00 | 26508 | .0471 | 1.00 | of P121 values |
| 2 | 27150.00 | | | 1.00 | |
| 3 | 26135.00 | | | 1.00 | |
| 4 | 25462.00 | | | 1.00 | |
| 5 | 26896.00 | | | 1.00 | |
| 1 { 6 | 22094.00 | 22048 | .033 | .037 | 89 |
| 7 | 22002.00 | | | 1.00 | |
| 2 { 8 | 22874.00 | 22955 | .035 | .033 | 106 |
| 9 | 23036.00 | | | 1.00 | |
| 3 { 10 | 21345.00 | 22335 | .034 | .032 | 106 |
| 11 | 23325.00 | | | 1.00 | |
| 4 { 12 | 17420.00 | 17637 | .027 | .029 | 93 |
| 13 | 17853.00 | | | 1.00 | |
| 5 { 14 | 19189.00 | 19840 | .030 | .031 | 91 |
| 15 | 20491.00 | | | 1.00 | |
| 6 { 16 | 14064.00 | 14229 | .021 | .027 | 78 |
| 17 | 14394.00 | | | 1.00 | |
| 7 { 18 | 19638.00 | 20655 | .031 | .033 | 94 |
| 19 | 21673.00 | | | 1.00 | |
| 8 { 20 | 22693.00 | 20245 | .031 | .031 | 89 |
| 21 | 17798.00 | | | 1.00 | |
| 9 { 22 | 17031.00 | 18271 | .028 | .031 | 90 |
| 23 | 19511.00 | | | 1.00 | |
| 24 | 804.00 | | | .022 | 0 |
| 25 | 710.00 | | | 1.00 | |
| 11 { 26 | 17770.00 | 18729 | .028 | .032 | 88 |
| 27 | 19687.00 | | | 1.00 | |
| 12 { 28 | 166725.00 | | | .054 | |
| 29 | 170523.00 | | | 1.00 | |
| 13 { 30 | 19772.00 | 19521 | .030 | .034 | 97 |
| 31 | 20070.00 | | | 1.00 | |
| 14 { 32 | 21891.00 | 19376 | .029 | .035 | 85 |
| 33 | 16862.00 | | | 1.00 | |
| 15 { 34 | 24156.00 | 22789 | .034 | .032 | 106 |
| 35 | 21422.00 | | | 1.00 | |
| 36 | 1454.00 | | | 1.00 | |
| 21 { 37 | 134586.00 | | | 1.00 | |

To Page No. _____

Read & Understood by me,

Michael P. [Signature]

Date

5/1/95

Invented by

[Signature]

Date

4/11/95

Recorded by

176

Project No. _____

Book No. _____

TITLE

Crack TFI same as P 171

From Page No. _____

These cells were grown for post induction

cells are 9504-10-767-03-003 R

grown 4-11-95

resuspended 110 g cells in 440 ml (at room
 ext buffer (P167, 3) but no detergent
 10,000 PSI on minigolfer, 1 pass
 Bring to 75°C in 90°C water (~10 min)
 75°C for 15 min more.
 cool in ice slurry

Add NaCl to 200 mM Cf

Fr I vol = ~~510~~ 510 ml
 so add 5.96 g NaCl

add PEI (5% stock pH 7.4) to Cf = 0.

(used 0.40% last time (P 171) but want to get as
 as much DNA as possible

add 50.4 ml 5% PEI to 510 ml Fr I + N.

⇒ Cf = 0.45%

add PEI dropwise and
 stir 15 min more

spin GSA 13,000 RPM 30'

recovered 495 ml sup (= Fr I' / 1

Witnessed & Understood by me,

Date

Invented by

Date

To Page N

D. Damerica Polans

5/1/95

Record d by

4-13-95

P116 continued
Experiment done on P. 123

Pr j ct N . _____ Exhibit 70
Book No. _____ Appl. No. 09/558,421

117

ge N

Still Needed 3

cut with Ord I to see if full length lac Z is present
(assuming either AFI III or Aat II recognition sequence
had a point mutation generating). There are the "410" and "465 bp

miniprep # 54, 58, 64, 73, 87, 98, 103, 108, 113, 125

plus Aat II, AFI III

cut with 55+ I to see if R1 site in MCS was
a point mutation (or very small deletion
(see on P(07 at bottom) resulting in the "goomers"

miniprep # 3, 29,

Recut with 17 µl miniprep and load 30 µl?

25 µl reaction
to try to resolve the "No results"

miniprep # 20, 39, 71, 74, 75, 76

To Page No. _____

ed & Understood by me,

me a Bolanos

Date

2/16/95

Invented by

Recorded by

Date

1-31-95

Project No. _____

Book No. _____

TITLE

Sephacryl

200

178

From Page No. _____

resuspended entire Am. S₂ pellet in buffer B (P.
added 3 ml to ~ 1 ml pellet.
tributyl

spin SS34, 13 K RPM, 5 min

odd ~ 20 μ l buffer B \pm pellet
respin \rightarrow 20 μ l buffer B more

need to microfuge 15 min to clarify

$V_f = 3.5$ ml ($\approx 1.9\%$ of 180 ml G100 col)

Load on 180 ml sephacryl 200

elute with $\frac{1}{2}$ col vol/hr buffer B (ie 1.5 ml/min)

note mol started coming off
column ~ 98 ml

98 ml / 180 ml col vol $\approx 54\%$ col vol

To Page N. _____

Inness d & Und rsto d by me,

Deanna Polans

Date

5/1/95

Invented by

Record d by

Date

4-18-95

PAGE 179 OF NOTEBOOK WAS BLANK

Project No. _____

Book No. _____

TITLE

Standard
TFI unit assay

From Page No. _____

mix used by epicenter same as tag unit assay (P125)
except only 160 μ g DNA/ml instead of 500 μ g
in Rxn

0.5 M TAPs pH 9.3
1 M MgCl₂
3 M KCl

A5500 μ l60 μ l500 μ lV_T = 2,060 μ l

"TFI Rxn mix"

A229 μ l

✓

10 mM dNTP

66.7 μ l

✓

3.7 mg/ml DNA

144.2 μ l

✓

10 mM γ -³²P-ATP6 μ l

✓

H₂O

2754

3.2 μ luse 48 μ l / 50 μ l reaction

To Page N

Witnessed & Understood by me,

Deborah Polansky

Date

5/1/95

Invented by

Recorded by

Date

4-17-95

ag N — Tube # 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
 Running p180 4Pul —————→
 PEI 1/100 dil 2λ
 resuspended 1/10000 dil 2λ
 3xyl w/ fractions 1/1000 dil 2 μl —————→
 # [4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20]
 14/1 100 dil 2 —
 18095 V_f = 50 μl, 74°C, 10 min 2λ 1/100
 18094 tube

21 1/100
Am 504 sup
(2.4 m m
Am 504 in
section)

| | SAM | CPM1 | pmol | u/pl | total units |
|-------|-----|----------|-------|----------|---|
| PEI | 1 | 4110.00 | 163 | 2.45 | [1.21 x 10 ⁶ units in 495 ml] |
| yeast | 2 | 6087.00 | 562.9 | | |
| 4 | 3 | 308.00 | | | fraction
pool 7-12
= 1.8 ml total |
| 5 | 4 | 356.00 | | | |
| 6 | 5 | 678.00 | | | |
| 7 | 6 | 3373.00 | | | |
| 8 | 7 | 8181.00 | | | |
| 9 | 8 | 11817.00 | | | |
| 10 | 9 | 9111.00 | | | |
| 11 | 10 | 8925.00 | | | |
| 12 | 11 | 5943.00 | | | |
| 13 | 12 | 2583.00 | | | |
| 14 | 13 | 1385.00 | | | |
| 15 | 14 | 773.00 | | | |
| 16 | 15 | 351.00 | | | |
| 17 | 16 | 299.00 | | | |
| 18 | 17 | 304.00 | | | |
| 19 | 18 | 245.00 | | | |
| 20 | 19 | 407.00 | | | |
| 21 | 20 | 2651.00 | 105 | 1.58 | (expected only 1 u/l in stock from epicentre) |
| 22 | 21 | 358.00 | | | |
| 23 | 22 | 818.00 | | | |
| 24 | 23 | 60259.00 | 37.7 | cpm/pmol | |

105 1.58 (expected only 1 1/2 in stock from epicenter)

37.7 cpm/pmol

for fr ⁷⁻¹²~~6~~ = average of ~8000 cpm for 1 Pml

$$\Rightarrow 47.7 \text{ u/ml} \Rightarrow \underline{1859,000 \text{ total units / 18 ml}}$$

or $\sim 72\%$ recovery from FrI'/PEI

182

Project No. _____

Book No. _____

TITLE

Blue sepharose

From Page No. _____

load pooled sepharose 200 fractions #7-12 (18 ml V to
on 20 ml Blue at 0.35 ml/min (~1 col vol)
wash 5 col vol O/N at 0.16 ml/min buffer
gradient is 400 ml vol 50 mM - 1 M KCl
(use buffer B-C p162)
at 3 col vol/hr = 1 ml/min, 6 ml fraction

Buffer

1M Tris pH 7.5
0.5M EDTA
Glycerol
3ME
KCl

* D

200 ml ✓✓
1.6 ml ✓✓
640 ml ✓✓
2.8 ml ✓✓
29.8g ✓✓

PL

(50 mM KCl)

E

25 ml 12.5 ✓✓
0.2 ml 0.1 ✓✓
80 ml 40 ml ✓✓
350.2 ml 1175 ✓
142g 74.5g ✓

1E

500 ml

2M KCl

(note buffer D is
75 mM KCl in Tag Pop 91342)
but only 50 mM here)

enter "2" to set to bank 2
then 5

HOD 5 BANK 2

.00 CONC 1B 0 0
.00 CONC 2B 0 0
.00 ML MIN 1
00 F 1

400
400

still 1

a

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Date

5/1/95

Invented by

Recorded by

Date

4-19-95

To Page N

ge N — pool Blue fractions 24-32 based on UV profile

$V_f = 54 \text{ ml}$

Dialyze against 5 L buffer D (PIB2) O/N
 recovered ~ 60 ml

Conductivity

10 μl in 1 ml H_2O

buffer D
 in cell effluent
 to equilibration O/N

101 μS = 10.1 mS
 98 μS = 9.8 mS

Dialysate

99

9.9 mS

(can see P 41 where results are similar)
 for Toq

To Page No. _____

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maea Polansky

Date

5/1/95

Invented by

Recorded by

Date

4-20-95

PAGE 184 OF NOTEBOOK WAS BLANK

Heparin AF (20ml vol)

Pr j ct No. _____
Bo k N . _____

185

EN - Equilibrate O/N with buffer D, P182 (50 mM KCl) → see P183 for conductivity of col effluent
Load ~ 60 ml dialysate (P183) at 0.67 ml/min
= 2 col vol/hr (as done on P11 for rTag)
(1 min/min)

wash ~ 1 col vol 0.67 ml/min

for gradients want to make it fairly flat for first try
of TPI on Heparin.

Gradient:

50-700 mM KCl (= 0-35% pump B since
E is 2 M KCl)

20 col vol = 400 ml, 4 ml/hr (so 100 fractions total)

run at 2 col vol/hr

so need 10 hours for whole gradient

rTag comes off Heparin ~ 400 mM KCl (see P 46)

so might see TPI ~ 6 hr post start ~ late afternoon
if TPI same as Tag

(loading done ~ 10:25 AM wash 30 min (= 1 col vol)
gradient start ~ 11 AM

IOD 5 BANK 1

.00 CONC AB
.00 CONC AB
.00 ML/MIN
.00 PORT.SET
.00 PORT.SET
.00 VALUE.POS
.00 VALUE.POS
.00 CONC AB
.00 ML/MIN

To Page N . _____

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reana Polarp

Date

5/1/95

Invented by

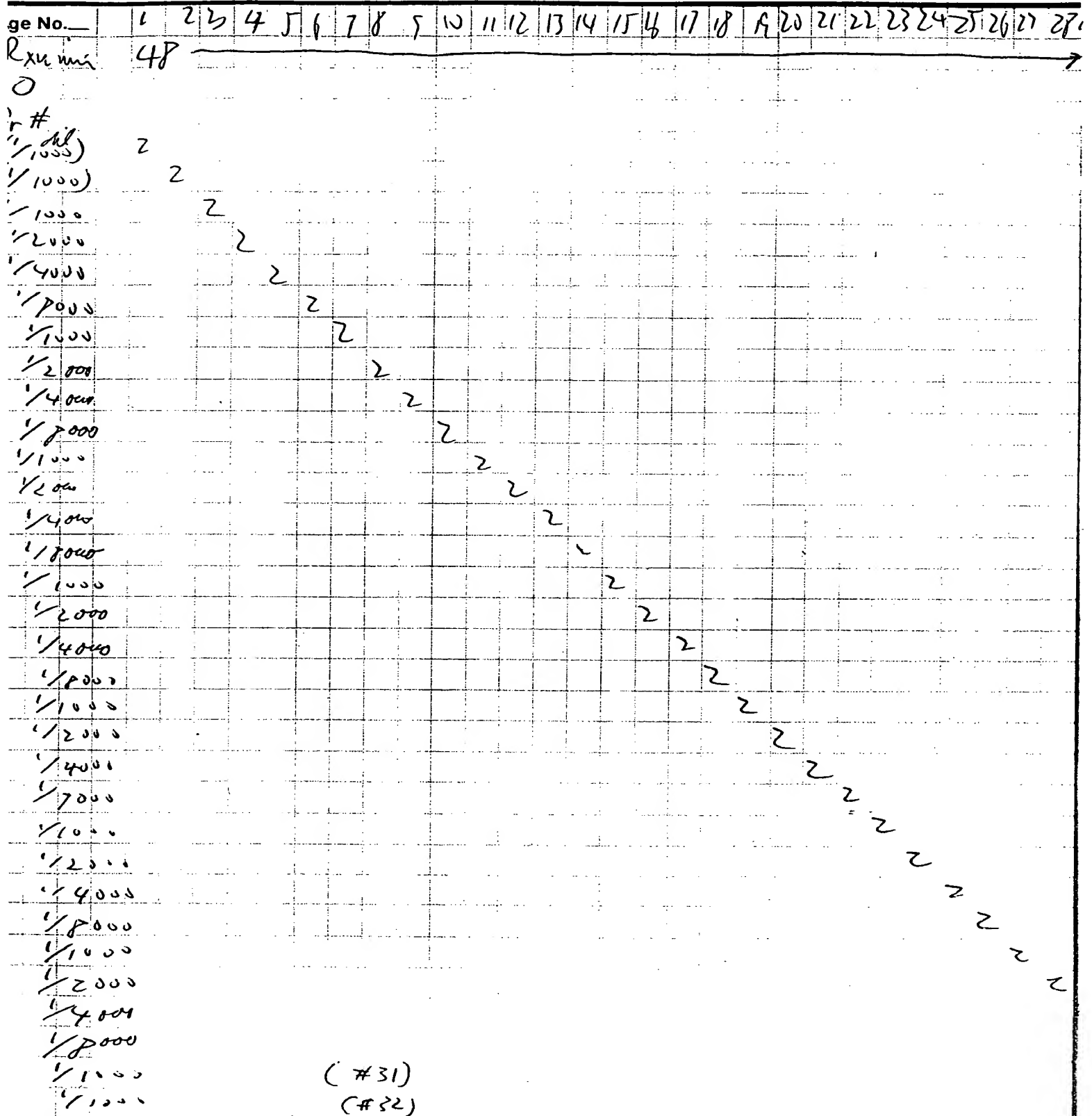
R cord d by

Date

4-20-95

141
 pol assay Hepain fractions

Exhibit 74
 Project N. _____ Appl. No. 09/558,421
 Book No. _____



cool R 24-32 P173 1/1000 (#31)
 dialysis P173 1/2000 (#32)
 1/1000 (#33, 34)
 1/2000 (#35, 36)

To Page No. _____

| | | | |
|---|----------------|-----------------|-----------------|
| Read & Understood by me,
<i>Michael Polansky</i> | Date
5/1/95 | Invented by
 | Date
4-21-95 |
| | Recorded by | | |

Project No. _____

Book No. _____

TITLE _____

From Pag No. _____

| | | | <u>pmol</u> | <u>u/pl</u> | <u>average</u>
<u>u/pl</u> | <u>fraction</u> | <u>total</u>
<u>units</u> |
|----|----|---------|-------------|-------------|-------------------------------|--------------------------------|------------------------------|
| 38 | 1 | 321.00 | 12 | | 1.9 | | |
| 39 | 2 | 526.00 | 20 | 7.0 | 3.0 | | |
| 40 | 3 | 1566.00 | 60 | 9.03 | 9.9 ave | | |
| | 4 | 928.00 | | 10.7 | | 4ml | 39600 |
| | 5 | 513.00 | | | | | |
| | 6 | 326.00 | | | | | |
| | 7 | 3904.00 | | 22.52 | | | |
| | 8 | 1849.00 | | 21.35 | 21.9 ave | | |
| 41 | 9 | 1346.00 | | | | 4ml | 87600 |
| | 10 | 792.00 | | | | | |
| | 11 | 5730.00 | | 33 | | | |
| 42 | 12 | 3486.00 | | 40 | 40.5 | 4ml | 162000 |
| | 13 | 1668.00 | | 37 | | | |
| | 14 | 1117.00 | | 51 | | | |
| 43 | 15 | 5064.00 | | 23 | | | |
| | 16 | 3156.00 | | 36 | 41.3 | 2.68ml | 110684 |
| | 17 | 1890.00 | | 43 | | | |
| | 18 | 1239.00 | | 57 | | | |
| 44 | 19 | 6029.00 | | 34 | | | |
| | 20 | 3974.00 | | 43.8 | 43.8 | 2.68 | 117384 |
| | 21 | 2233.00 | | 51 | | | |
| | 22 | 969.00 | | 44.7 | | | |
| | 23 | 4489.00 | | 25.5 | | | |
| 45 | 24 | 2775.00 | | 25.5 | 35.6 | 2.68 | 95408 |
| | 25 | 1960.00 | | 25.5 | | | |
| | 26 | 858.00 | | 29 | | | |
| 46 | 27 | 2156.00 | | 12.4 | 12.3 | 2.68 | 32964 |
| | 28 | 1056.00 | | 12.1 | | | |
| | 29 | 843.00 | | | | | |
| | 30 | 364.00 | | | | | |
| 47 | 31 | 847.00 | | | 4.9 | | |
| 48 | 32 | 465.00 | | | 2.7 | | 13000 |
| 49 | 33 | 4246.00 | | | | | |
| 50 | 34 | 2441.00 | 93.9 | 14.0 | | 54ml | 756000 |
| 51 | 35 | 3795.00 | | | | 60ml | |
| 52 | 36 | 2266.00 | | 13.9 | | off dialysis | 751000 |
| 53 | 37 | 165.00 | | | | 30 100% recovery from dialysis | |

32.5 CFU/pmol

(accidentally omitted
47 pl as indicated
pmol fraction)

Witnessed & Understood by m ,

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Dat

4-21-95

To Page 1

| Page N _____ | units | % recovery |
|----------------|-----------|------------|
| C'/PEI | 1,210,000 | 100% |
| monium sulfate | 1,280,000 | 100 |
| phacryl 200 | 859,000 | 71 |
| sepharose | 756,000 | 62 |
| alysis | 751,000 | 62 |
| arin AF | 666,000 | 55 |

To Page No. _____

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Date

Invented by

Date

Sandra Polansky

5/1/95

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4-21-95

190

Project No. _____

Book No. _____

TITLE

grow λ pl, sp6 plasmid
in host lacking TFI sp6 plas
but containing Tet resistance
plasmid with λ promoter and
sp6 gene

From Page No.

Tet stock

LB 50ml
Tet 30 μ g/ml
add stab of cells \rightarrow 30°C shaking O/N
got no growth O/N! 4-24
 \rightarrow no it just grew
slow - had cells 36 hr

Repeat with m. longi Tet and
at only 15 μ g/ml
after 36 hr) grow O/N at 30°C
(including stock used above that &
should dissolve "crystalline" TC in E
made fresh ~~Tet~~ TC stock

139 mg Sigm. No. T 3258 Tetracycline
a little water
(it still doesn't go into solution)

100% ETOH (good stuff from Cor. Nor.)
up to 27 ml
= 5 mg/ml store in foil, -20°C

inoculate 1 ml of ON #5 into 50 ml
can circle grow + ~~45 μ g/ml~~ of fresh TC stock
shake at 30°C 30 μ g/ml

start 8:30 stop 3 PM got 0.22 mg cells
so add 0.88 ml Tag ext buffer (P167,3) for 0.2 μ g/ml

Witnessed & Understood by me,

Deena a Polay

Date

5/1/95

Invented by

Recorded by

Date

4-23-95

To Page N

Regeneration of columns

Project No. _____

Exhibit 76

Appl. No. 09/558,421

Book No. _____

191

e No. _____

Blue sepharose

2 col vol 6M Guanidinium HCl
5 col vol H₂O (immediately)
2 col vol 20% EtOH for storage

Heparin Af

2 col vol 4M urea
2 col vol H₂O
2 col vol 20% EtOH

(Co. 3 = 0.5M NaOH recommended)

sepharose S200

1/2 - 1 col vol 0.4M NaOH

contact with col = $\geq 1 \text{ hr} \leq 2 \text{ hr}$.

H₂O 2 col vol

20% EtOH for storage

run 0.4M NaOH at 2 ml/min
for 45 min (= 1/2 col vol)

(start 10:20am) H₂O for 3 hr at 2 ml/min
= 2 col vol and NaOH only in
contact with column for
45 min + 90 min

20% EtOH 3 hr 0.2 ml/min O/N

To Page No. _____

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Date

Invented by

Date

revised by

5/1/95

Recorded by

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Project No. _____

Book No. _____

TITLE

SDS gel for TFI prep

192

From Page No. _____

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

DH10BRL

Fr I P190

Fr I' (75°C, 30) P190

3λ

30λ

TFI fr I 2.45 u/l (P190)

3λ

fr I' 2.45 u/l (P190)

30λ

AmS04 resuspended

0.5

362 u/l

5200 u/l

3.5

Blue pool Fr 2432

12.5

14 u/l (P17P)

Heparin Fr #

39

3 u/l

5

40

9.9

5

41

21.9

5

42

40.5

5

43

41.3

5

44

43.7

5

45

35.6

5

46

12.3

5

47

4.9

5

TFI epimorph 1 u/l

30

cut TF31010A-502

2X sample buffer

30

H₂O

27-27-3027 17.525

load 15 μl MW standards

CTI cut 10064-012

run at ~29 mA

started 9:15 AM

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Witnessed & Understood by me,

Deena R Polamp

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5/1/95

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4-25-95

47 cm Heparin AF column

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proved a 1.5 cm x 47 cm (80ml) column
to try to separate the 2 peaks on P186-187, 9
flow rate is 0.204 ml/min by gravity.

gradient will be 50 mM - 400 mM
and 10 col vol = ~~1600 ml~~ 1600 ml
so gradient 1/2 as steep as P185, 9: 20ml col

pool fr 40-43 (14.7 ml total)
of Heparin (see P185-192, 9)

Dialyze ON against 1 L buffer D

(frms are ~ 300 mM KCl
so expect ~ 4.2 mM + 50 mM in buffer D)
start gradient ~ 9:30 AM
gradient is

1600 ml (20 col vol)
50 mM - 400 mM KCl (was 50 mM - 700 mM)

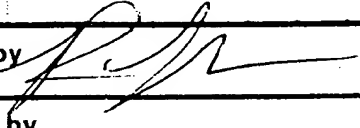
2 ml in 5 ml/min, so ^{13.3} 13.3 hr for gradient
4.5 hr/min / frn = 9 ml / frn (20 frms total)

note 1.5 ml/min gave only 0.2 mPa (column
is definitely running with backpressure) but
2 ml/min still only ~ 0.2 mPa so will
use 2 ml/min \Rightarrow 1.5 col vol/hr
used 2 col vol/hr for 20ml col P185, 9

To Page No. _____

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4-27-95

From Page No. _____

expect protein to start coming off at $\sim 65\%$ of the
gradient is $\sim 1040 \text{ ml} = 8.7 \text{ hrs.}$
or $\sim 6:30 \text{ pm}$

since pol started coming off at $13\% \text{ } \phi + 50 \text{ mm}$
 $= 410 \text{ mm}$

Comparison of 80 and 20 ml columns

| | | |
|---------------|-----------------------------|-----------------------------|
| col vol | 20 ml | 80 ml |
| col height | 11 cm | 47 cm |
| gradient vol | 20 col vol | 20 col vol |
| gradient stop | <u>35 mm KEE</u>
col vol | <u>20 mm KEE</u>
col vol |
| flow rate | 2 col vol/hr | 1.5 col vol/hr. |

Therefore the new col is $4\times$ longer has $0.75\times$
flatter gradient and is $0.75\times$ slower flow rate
so hope to get better separation of 2 peaks see
on p 186-187, 9

THOD 5 BANK 2

1.00 CONC XB C
1.00 CONC XB C
.00 ML/MIN 2.
.00 PORT.SET 3
.00 PORT.SET 6
.00 VALVE.POS 1
.00 VALVE.POS 2
0.0 CONC XB 20
0.0 ML/MIN 0.1

Witness d & Und rsto d by me,

Deeann Polarp

Date

5/1/95

Invented by

Recorded by

Date

4-27-95